Exhibit A

International Immunology, Vol. 11, No. 1, pp. 11–20

Minimal cross-linking and epitope requirements for CD40-dependent suppression of apoptosis contrast with those for promotion of the cell cycle and homotypic adhesions in human B cells

John D. Pound, Anita Challa, Michelle J. Holder, Richard J. Armitage[1], Steven K. Dower[1,4], William C. Fanslow[1], Hitoshi Kikutani[2], Staffan Paulie[3], Christopher D. Gregory and **John Gordon**

Division of Immunity and Infection, University of Birmingham, Edgbaston, Birmingham B15 2TT, UK
[1]Immunex Corp., Seattle, WA 98101, USA
[2]Department of Molecular Immunology, Research Institute for Microbial Diseases, Osaka University, Osaka 565, Japan
[3]Mabtech AB, SE-131 40 Nacka, Sweden

[4]Present address: Department of Medicine and Pharmacology, University of Sheffield Medical School, Sheffield SI0 2JF, UK

Keywords: B cell, receptor, CD40 ligand, germinal center, signalling

Abstract

Eight different CD40 mAb shared with soluble trimeric CD40 ligand (sCD40LT) the capacity to rescue germinal center (GC) B cells from spontaneous apoptosis and to suppress antigen receptor-driven apoptosis in group I Burkitt's lymphoma cells. Three mAb (G28-5, M2 and M3) mimicked sCD40LT in its ability to promote strong homotypic adhesion in resting B cells, whereas others (EA5, BL-OGY/C4 and 5C3) failed to stimulate strong clustering. Binding studies revealed that only those mAb that promoted strong B cell clustering bound at, or near to, the CD40L binding site. While all eight mAb and sCD40LT were capable of synergizing with IL-4 or phorbol ester for promoting DNA synthesis in resting B cells, co-stimulus-independent activation of the cells into cycle through CD40 related directly to the extent of receptor cross-linking. Thus, mAb which bound outside the CD40L binding site synergized with sCD40LT for promoting DNA synthesis; maximal levels of stimulation were achieved by presenting any of the mAb on CD32 transfectants in the absence of sCD40LT or by cross-linking bound sCD40LT with a second antibody. Monomeric sCD40L, which was able to promote rescue of GC B cells from apoptosis, was unable to drive resting B cells into cycle. These studies demonstrate that CD40-dependent rescue of human B cells from apoptosis requires minimal cross-linking and is essentially epitope independent, whereas the requirements for promoting cell cycle progression and homotypic adhesion are more stringent. Possible mechanisms underlying these differences and their physiological significance are discussed.

Introduction

CD40 and its ligand (CD40L) are now established as being central to the development of T-dependent B cell responses. For resting B cells, co-engagement of CD40 and antigen receptor results in a lowering of the threshold for triggering through the latter by two orders of magnitude (1). Resting B cells rapidly form large aggregates on CD40 signalling via both LFA-1-dependent (2) and -independent mechanisms (3). IL-4-dependent CD23 production is dramatically augmented on engaging CD40 (4) and the CD40–CD40L interaction provides a permissive cognate signal for IL-4-driven switching to IgE synthesis in naive B cells (5). The selection process occurring in germinal centers (GC) which results in affinity maturation of

Correspondence to: J. Gordon

Transmitting editor: G. Klein

Received 31 March 1998, *accepted* 21 September 1998

the immune response to thymus-dependent antigens following hypermutation on Ig *V*-region genes is CD40 dependent: antigen-rescued centrocytes require cognate interaction with T_h cells containing preformed CD40L for their long-term survival and recruitment into a memory pool (6). Neoplastic phenotypic equivalents of GC B cells, as represented by group I (biopsy-like) Burkitt's lymphoma (BL) cell lines, can be effectively rescued from activation-induced programmed death on engaging cell surface CD40 (7).

The diverse functional outcome to CD40 ligation on the various subpopulations may reflect a differential coupling to intracellular signal transduction pathways during B cell development: we have termed this process 'CD40 receptor rewiring' (8). Thus, for example, whereas there is little tyrosine kinase activity stimulated in resting B cells on engaging CD40, extensive phosphorylation of multiple substrates on tyrosine residues has been reported following CD40-dependent stimulation of GC B cells (9). The ultimate response engendered on CD40 ligation may also depend on the degree of receptor cross-linking. Thus, while for both resting and GC B cells ligand in soluble form (either as a mAb or as trimeric CD40L) can promote certain phenotypic changes without significantly influencing proliferation, the multivalent presentation of ligand on a cell surface membrane can drive or maintain active cell cycle in both populations (10). Finally, to account for its multifunctional role, there is the consideration of possible multiple ligands for CD40 as has been described for the nerve growth factor receptor (NGFR) (11) and the tumour necrosis factor receptor (TNFR) (12), which are members of the same receptor family. Comparative functional studies with limited numbers of mAb to CD40 have indicated that different outcomes can be engendered depending upon epitope specificity of binding (13,14). Evidence for a second CD40L has been reported although formal identification through its cloning and sequencing is still awaited (15).

The present study examines critically the extracellular signalling requirements for promoting the diverse phenotypic changes which are seen to occur in resting B cells, GC B cells and neoplastic phenotypic equivalents of the latter, on ligating CD40. Detailed analysis with eight different mAb to CD40 and with CD40L itself reveals that suppression of apoptosis in GC B cells and the promotion of homotypic adhesions or entry into the cell cycle of resting B cells have markedly distinct requirements. Possible implications of these findings to B cell physiology are discussed.

Methods

Reagents

Mouse monoclonal CD40 antibodies BL-OGY/C4, EA5, 5C3, S2C6, G28-5, HB14, M2 and M3 were from the panel submitted to the Fifth International Workshop on Human Leukocyte Typing (16,17). They were IgG1 isotype with the exception of BL-OGY/C4 (IgM). BU1 (mouse IgG2a anti-μ mAb) and BU25 (mouse IgG1 anti-MHC class II) were produced in the Department of Immunology, Birmingham University. Mouse IgG1 anti-CD40L mAb, M79 and M91 were produced by Immunex Corp. (18). Rabbit anti-human IgM coupled to Sepharose beads (BioRad, Richmond, CA) was employed in B cell proliferation experiments.

Murine CD40LT, human CD40LT and human monomeric CD40L were produced as described in Fanslow *et al.* (19), and the human CD40–IgGFc fusion protein (CD40–Fc) as in Fanslow *et al.* (20).

Cells and cell culture

Human tonsillar GC B cell and resting B cell fractions were isolated as previously described (21). Resting tonsillar B cell fractions were >95% $CD19^+$, >70% IgD^+ and ≤2% $CD3^+$, and GC B cell fractions were >93% $CD19^+$, >76% $CD38^+/IgD^-$, >64% $CD77^+$ and ≤3% $CD3^+$ as defined by flow cytometry on a Becton Dickinson FACScan (Becton Dickinson, Cowley, UK) using FITC- and phycoerythrin (PE)-conjugated mAb (Becton Dickinson and Dako, High Wycombe, UK).

Human peripheral blood mononuclear cells (PBMC) were purified from the blood of healthy donors by centrifugation over Histopaque (Sigma, St Louis, MO). B cells were isolated from PBMC by depletion of cells rosetting with aminoethylisothiouronium bromide (AET)-treated sheep red blood cells (SRBC) and treatment of remaining cells with B cell Lympho-kwik (One Lambda, Los Angeles, CA) for 1 h at 37°C to lyse contaminating non-B cells. The resulting B cell population was >98% $CD20^+$ with no detectable $CD3^+$ T cells as detected by flow cytometry. T cells were purified by recovery of cells rosetting with AET-treated SRBC, lysis of SRBC, and removal of residual B cells and monocytes by plastic adherence.

Cells were cultured at 37°C in a humidified incubator in 5% CO_2/95% air. Culture medium (CM) was RPMI 1640 containing penicillin (100 IU/ml)/streptomycin (100 μg/ml), 2 mM glutamine (Gibco), and 10% (v/v) FCS (Advanced Protein Products, Dudley, West Midlands, UK). B cells ($0.4–2\times10^6$/ml) were cultured in triplicate wells of flat-bottom 96-well microtitre plates (Becton Dickinson Labware, Oxford, UK) in a total volume of 100 or 200 μl/well. The Epstein–Barr-negative group I BL cell line, L3055, was maintained in CM containing prescreened FCS. Mouse L cells transfected with the gene for human CD32 (CD32-L cells) were obtained from DNAX Research Institute (Palo Alto, CA). CD32-L cells were cultured in HAT selection medium consisting of CM containing hypoxanthine (0.1 mM), aminopterin (0.4 μM) and thymidine (16 μM) (Sigma). The adherent CD32-L cells were recovered using 0.02% (w/v) EDTA in PBS (pH 7.0) and resuspended in CM. They were γ irradiated with a dose of 20,000 rad before addition to B cell cultures at a ratio of (B cells:L cells) 10:1.

Competitive binding studies

Inhibition of binding of soluble CD40 to CD40L on T cells. Peripheral blood T cells were cultured with phorbol myristate acetate (PMA; Sigma; 10 ng/ml) and ionomycin (500 ng/ml) (Calbiochem) for 18 h in order to stimulate surface expression of CD40L. Biotinylated CD40–Fc (1 μg/ml) was pre-incubated with CD40 mAb (10 μg/ml) or control mouse monoclonal IgG for 30 min and then incubated with the activated T cells for 30 min. Bound CD40 was revealed by development with streptavidin–PE (Becton Dickinson Mountain View, CA). Background mean fluorescence intensity (MFI) was determined using biotinylated IL-4 receptor–Fc as control Fc protein and

streptavidin–PE. All binding reactions were performed at 4°C in the presence of 0.02% w/v sodium azide.

Inhibition of binding of CD40 mAb S2C6 to CD40 on B cells

Resting tonsillar B cells were incubated with CD40 mAb (10 μg/ml) or MHC class II mAb BU25 (10 μg/ml) for 40 min and then washed with PBS containing 0.1% sodium azide (PBS/azide). Cells were then incubated with biotinylated S2C6 (100 μg/ml) and then washed in PBS/azide. Binding of S2C6 was visualized by incubation of cells with FITC-conjugated streptavidin (Sigma) and, after further washing, quantified by flow cytometry. All incubations were performed for 40 min on melting ice. The percentage binding inhibition was calculated as for CD40–Fc binding to T cells except that background was determined from the MFI of cells incubated without conjugated S2C6.

Surface plasmon resonance (SPR)

SPR studies of CD40–CD40L–CD40 mAb interactions were performed using a BIAcore biosensor (Pharmacia, Piscataway, NJ). All experiments were performed using an indirect immobilization protocol as described in detail in Arend *et al.* (22). Biosensor chips were coupled with a high avidity goat anti-human IgG1 antibody (GaHIg) (Jackson, Baltimore, MD) using the standard amine-coupling kit according to the manufacturer's recommendation. Briefly, chips were activated with a 6 min pulse of *N*-hydroxy succinimide/1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride at 3 ml/min followed by a 6 min pulse of antibody (50 μg/ml) at 3 ml/min in 10 mM sodium acetate, pH 5.0. Unreacted coupling reagent was blocked with a 6 min pulse of ethanolamine at 3 ml/min. The antibody-coated chips were coated with CD40–Fc fusion protein (35 μg/ml) using a 13 min pulse at the same flow rate. This led to immobilization of ~500 pg/mm^2 of CD40–Fc with an essentially zero off rate. The anti-human IgG antibody was regenerated with a 2 min pulse of 1 M formic acid at 5 ml/min, which removed all the Fc fusion protein while causing a ~2% loss in the Fc binding activity of the antibody. For testing the effect of anti-CD40 antibodies on sCD40LT–CD40 interactions, chips coated with CD40–Fc–GaHIg complexes were incubated with antibodies with a 35 ml injection at 3 ml/min using 5 μg/ml of antibody, with the exception of G28-5 which was used at 2.5 μg/ml. These conditions routinely gave 90% occupancy (mol/mol) of the CD40–Fc by antibody based on a mol. wt of the CD40–Fc of 120,000. Subsequently the binding activity of the CD40–Fc–anti-CD40 complexes was tested by incubating the chips with murine sCD40LT at 1.2 μg/ml using a 35 ml injection at 3 ml/min. All data were corrected for binding of reagents to chips coated with GaHIg but no CD40–Fc, by substituting buffer only (20 mM HEPES, 0.15 M NaCl, pH 7.4) for the CD40–Fc fusion protein pulse. For the epitope mapping experiments all conditions were essentially the same except that the flow rate during the antibody injections was 2 ml/min.

All BIAcore data were analyzed by non-linear least squares fitting using the Marquandt–Levenberg algorithm. The data were utilized without transformation as relative units versus time, no weighting was applied. The equations used were of the general form:

$$\text{bound}_t = \Sigma_i \text{bound}_{\text{inf}i}[1 - \exp(-k_i t)]$$ for the association phase

and

$$\text{bound}_t = \Sigma_i \text{bound}_{0i}[\exp(-k_i t)]$$ for the dissociation phase

with the index running from 1 to N. $\text{bound}_{\text{inf}}$ is the amount bound at infinite time, bound_0 is the amount bound at zero time and k is the associated rate constant for the ith component. Data were fit with $N = 1$ then 2 then 3. In general $N = 2$ or 3 sufficed to produce a fit beyond which the sum of squares ceased decreasing, showing that further increasing model complexity was not necessary. These methods are essentially as described elsewhere (23). All analyses were programmed either in MLAB (Civilised Software) or in HiQ (Bimillenium). Data from the BIAcore were exported from the instrument background corrected and formatted prior to analysis using Microsoft Excel software.

DNA synthesis

DNA synthesis was determined by thymidine incorporation. After culture with CD40 mAb or sCD40L for the times specified in Results, cells were pulsed for 16–18 h with [^{3}H]thymidine (Amersham International, Amersham UK; 10 μCi/ml in CM, 50 μl/well) and harvested on a Skatron cell harvester (Helis Bio, Newmarket, UK). Assays were performed in triplicate.

Rescue from apoptosis

Spontaneous apoptosis of GC B cells after 24 h in culture was estimated by enumeration of intact and fragmented cells in Romanowski stained cytocentrifuge preparations (24). Percentage rescue from apoptosis was calculated as: [(% intact cells after culture with test reagent) – (% intact cells in control)/100 – (% intact cells in control)]×100.

L3055 cells were induced to undergo apoptosis by culture with anti-μ mAb BU1 (10 μg/ml) and rescue was determined after 24 h as described for GC B cells.

Homotypic adhesions

The extent of aggregation of resting tonsillar B cells was determined semiquantitatively by phase-contrast microscopy of cultures 48 h after addition of CD40 mAb or sCD40LT.

Results

Competitive binding studies and relative epitope mapping

When CD40 mAb were compared for their ability to inhibit the binding of a soluble bivalent CD40–Fc construct to CD40L expressed on activated T cells, a spectrum of activities was found (Table 1). G28-5, M2 and M3 were effective inhibitors suggesting that the epitopes recognized by these antibodies were within or overlapped with the CD40L binding site. EA5, HB14 and BL-OGY/C4 in turn were partial inhibitors, whereas 5C3 was exceptional in enhancing this interaction, indicating recognition by the latter of an epitope outside of and cooperating with the ligand binding site. Consistent with this, all of the CD40 mAb with the exception of 5C3 were able to block the binding of S2C6 to resting B cells (Table 1). G28-5, M2, S2C6 and EA5 were each found to reciprocally cross-block when their competitive binding to immobilized CD40–Fc was meas-

Table 1. Competition between CD40 mAb and CD40L for binding to CD40

	Inhibition (%)								
	BL-OGY/C4	EA5	5C3	G28-5	S2C6	HB14	M2	M3	Control
CD40L on T cells[a]	25	57 ± 24	−32 ± 30	90 ± 9	82 ± 8	37 ± 15	97 ± 3	96 ± 3	8 ± 3[b]
S2C6[c]	92	89	22	90	84	89	73	78	0[d]

[a]Inhibition of binding of biotinylated CD40–Fc (1 μg/ml) to CD40L expressed by activated T cells after pre-incubation with CD40 mAb (10 μg/ml). Values are means ± SD for three to seven experiments; mean of two experiments for BL-OGY/C4.
[b]Inhibition by monoclonal murine IgG (10 μg/ml).
[c]Inhibition of binding of biotinylated CD40 mAb S2C6 (100 μg/ml) to resting tonsillar B cells by pre-incubation of cells with CD40 mAb (10 μg/ml).
[d]Inhibition by MHC class II mAb, BU25(10 μg/ml).
For further details see Methods.

ured by SPR (Fig. 1). The effect of CD40 mAb on the interaction between CD40 and CD40L was also assessed by measuring the association and dissociation of sCD40LT from immobilized CD40–Fc by SPR. Again G28-5 and M2 were strong inhibitors of this interaction (Table 2). S2C6, EA5 and 5C3 were partial inhibitors however, EA5 and 5C3 also acted to accelerate both the association and dissociation of ligand and receptor (Table 2 and Fig. 2).

All CD40 mAb promote rescue of B cells from apoptosis but only those competing for the CD40L binding site induce homotypic adhesions

Those CD40 mAb which could strongly inhibit CD40L binding mimicked the effect of sCD40LT in inducing homotypic adhesion of resting B cells, whereas those which were poor inhibitors did not promote clustering (Table 3); these differences were seen reproducibly over several experiments with a range of mAb concentrations (up to 5 μg/ml: data not detailed). In contrast, all of the antibodies shared with sCD40LT the ability to rescue both GC B cells and BL cells from apoptosis (Table 3). For GC B cells this was assessed as suppression of their spontaneous apoptosis as judged by morphological criteria; for L3055 BL cells as rescue from the almost complete apoptosis promoted by the anti-IgM mAb BU1 assessed either morphologically or by the ability to restore the DNA synthesis otherwise inhibited as the cells enter growth arrest prior to undergoing apoptosis. Both assays have previously been shown to be robust markers for the detection of apoptosis occurring in normal GC B cells and the L3055 cell line (7,9,10,24,25).

CD40 epitopes can cooperate to stimulate DNA synthesis

All of the CD40 mAb and sCD40LT were effective in stimulating tonsillar resting B cells to low-rate DNA synthesis and acted synergistically with IL-4 or PMA to stimulate high-rate responses (Table 4). Those antibodies which were poor or partial inhibitors of CD40L binding (5C3, EA5, BL-OGY/C4, HB14 and S2C6) synergized with sCD40LT in promoting DNA synthesis in the absence of co-stimulants (Table 5), whereas the strong inhibitors (M2, M3 and G28-5) showed no evidence of this cooperative interaction. Paired combination of the majority of CD40 mAb in the absence of sCD40LT did not result in a synergistic or additive effect; however, stimulation of DNA synthesis by 5C3 was additive with the effect of mAb M2 and M3 (Table 5). The data sets shown in Tables 4 and 5 are representative of three similar experiments.

Evidence of enhanced stimulation occurring through co-ligation of distinct CD40 epitopes was also evident in peripheral blood B cells. For these cells, EA5 but not M2 was able to synergize with sCD40LT in promoting DNA synthesis (Fig. 3). Co-stimulation of the cells with IgM or IL-4 also revealed functional interaction between S2C6 [which partially blocks CD40L binding (Table 1)] and sCD40LT.

Co-factor-independent stimulation of DNA synthesis requires extensive cross-linking of CD40

When L cells transfected with CD32 were present in the cultures CD40 mAb were able to stimulate high-rate DNA synthesis by tonsillar resting or GC B cells regardless of epitope specificity (Fig. 4B and D). These responses were maximal as indicated by comparison with the effect of combined stimulation with PMA and ionomycin. They could not therefore be further enhanced by sCD40LT as was observed in the absence of CD32 transfectants (Fig. 4A and C). This suggests that co-factor-independent stimulation via CD40 can occur as a result of the extensive cross-linking facilitated by secondary interaction of mAb Fc regions with transfectant-bearing CD32. Similarly, peripheral blood B cells could be stimulated to high-rate DNA synthesis by sCD40LT in the presence of a CD40L mAb which recognized an epitope outside the CD40 binding site (and therefore able to cross-link CD40) but not by a mAb which blocked CD40 binding (Fig. 5). Again, these results are provided as representative data sets from several identical experiments.

Monomeric CD40L can rescue B cells from apoptosis but cannot stimulate DNA synthesis

A monomeric human sCD40L construct was found to be almost as effective as both the trimeric human sCD40LT and the dimeric G28-5 CD40 mAb in rescuing GC B cells from spontaneous apoptosis when used at equivalent concentrations (Fig. 6A). In marked contrast, monomeric sCD40L was unable to stimulate resting B cells into DNA synthesis, whereas the trimeric construct promoted sub-optimal, but still significant, DNA synthesis under otherwise identical conditions (Fig. 6B).

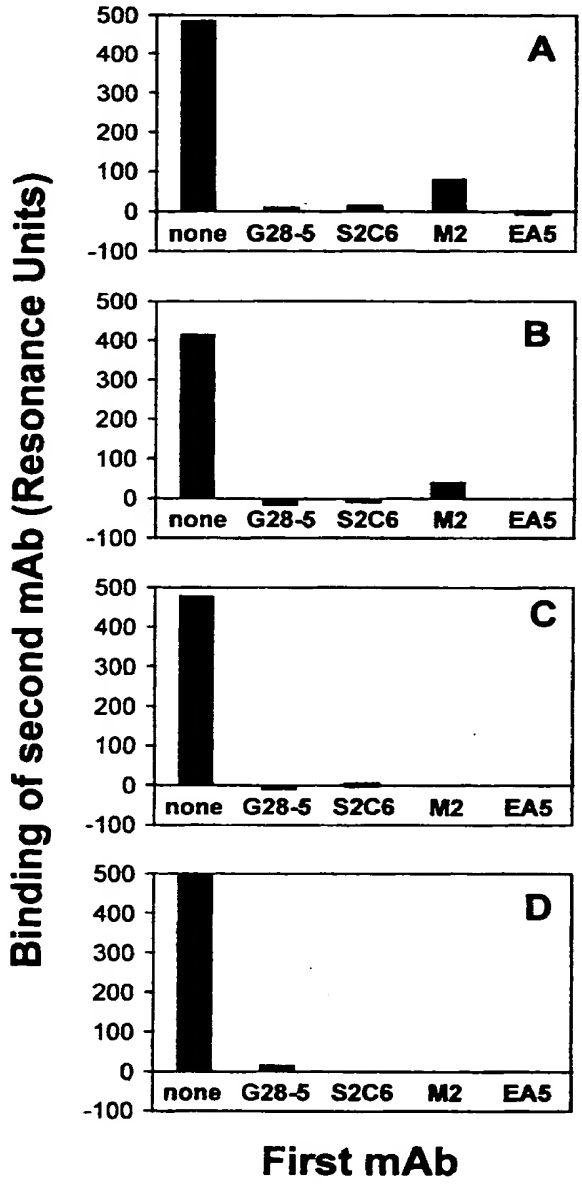


Fig. 1. Competition between CD40 mAb for binding to immobilized CD40–Fc. CD40–Fc was immobilized on a biosensor chip and a CD40 mAb allowed to bind until CD40–Fc was 90% saturated with the antibody. Each panel (A–D) shows the binding of a second CD40 antibody as determined by SPR. (A) EA5, (B) M2, (C) S2C6 and (D) G28-5. Antibodies were employed at a concentration of 5 μg/ml with the exception of G28-5, which was used at 2.5 μg/ml.

Table 2. Effects of CD40 mAb on the kinetics of binding of sCD40L trimer to immobilized CD40–Fc

	G28-5	S2C6	M2	EA5	5C3
Inhibition (%)[a]	75	44	100	49 ± 18[b]	34
Relative on rate[c]	ND	2	ND	12 ± 4[b]	13
Rapid dissociation (%)[d]	ND	10	ND	50 ± 22[b]	44

CD40–Fc was immobilized on a biosensor chip and the binding of sCD40LT in the presence of CD40 mAb was measured by SPR. All antibodies were employed at a concentration of 5 μg/ml and CD40LT at 1.2 μg/ml.

[a]Reduction in the amount of sCD40L bound relative to binding in the absence of mAb.

[b]Mean ± SD for three experiments.

[c]Ratio of the on rate in the presence of CD40 mAb to the on rate in the absence of mAb.

[d]Determined as described in Methods.

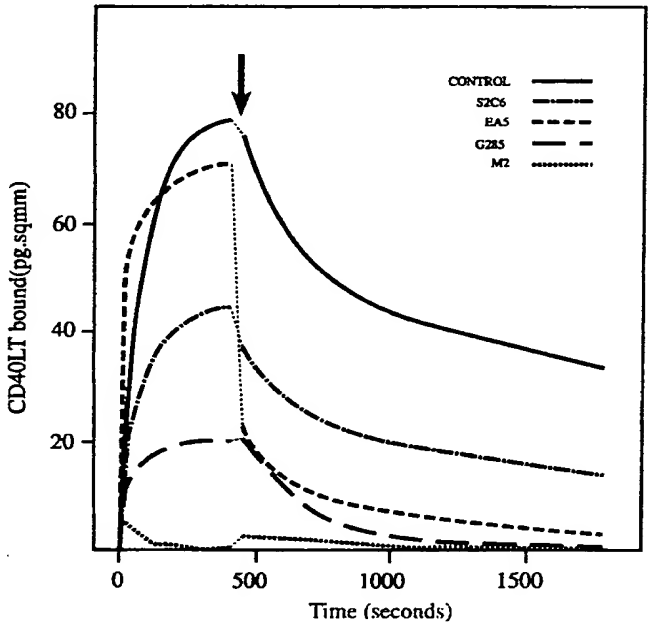


Fig. 2. Effect of CD40 mAb on kinetics of binding of sCD40L trimer to CD40–Fc. CD40–Fc was immobilized on a biosensor chip and the binding of sCD40LT in the presence of CD40 mAb was measured by SPR. Antibodies were employed at a concentration of 5 μg/ml with the exception of G28-5, which was used at 2.5 μg/ml; sCD40LT was used at 1.2 μg/ml. The arrow indicates the point at which CD40LT was replaced with buffer only in the injection.

Discussion

The present study demonstrates that, with respect to extracellular considerations, there are distinct requirements when signalling through CD40 for the induction of homotypic adhesions, the activation of cells into the cell cycle and the suppression of either spontaneous or induced apoptosis. The latter change in both GC B cells and a group I BL cell line could be engendered in an epitope-unrestricted manner by all eight mAb in soluble form (Table 3) and, as monomeric sCD40L could promote rescue of cells from apoptosis (Fig. 6), with presumably minimal cross-linking of the receptor. For resting B cells, epitope-dependent stimulation of homotypic adhesions via CD40 was apparent (Table 3) while entry into active cell cycle necessitated that the CD40 signal was delivered in an extensively cross-linked form (Figs 4 and 5). A model has been proposed for CD40–CD40L interaction (26) in which CD40 binds to two adjacent CD40L monomeric subunits, suggesting that extensive aggregation of the recep-

Table 3. Ability of CD40 mAb and sCD40L trimer to promote homotypic adhesion and rescue cells from apoptosis

	BL-OGY/C4	EA5	5C3	S2C6	G28-5	HB14	M2	M3	sCD40LT
Homotypic adhesion[a]	±	+	–	++	++++	++	++++	++++	+++
GC B cell rescue[b] (%)	49	69	54	20	51	60	69	49	74
L3055 cell rescue[c] (%)	87	74	77	78	86	80	80	86	80
L3055 cell DNA synthesis[d] (%)	76	70	58	72	85	75	78	80	84

CD40 mAb were added to cultures at 1 μg/ml. sCD40LT was added as a 1/10 dilution of culture supernatant from transfected COS cells.
[a]Homotypic adhesion in cultures of resting tonsillar B cells estimated after 48 h.
[b]Rescue as defined in Methods after 24 h in culture.
[c]As (b) in the presence of anti-μ mAb, BU1 (10 μg/ml).
[d][^{3}H]Thymidine uptake by L3055 cells after 24 h culture in the presence of anti-μ mAb, BU1 (10 μg/ml) expressed as a percentage of the value for cells cultured in the absence of BU1.
Data are means from three experiments except for homotypic adhesion which are representative of three experiments.

Table 4. Ability of CD40 mAb and sCD40L trimer to co-stimulate DNA synthesis in resting B cells.

	BL-OGY/C4	EA5	5C3	S2C6	G28-5	HB14	M2	M3	sCD40LT	CM
CM	3744	4098	3164	4554	5180	2812	4968	5019	4732	422
IL-4	15,943	12,707	14,822	23,160	28,801	18,427	26,944	24,537	32,004	645
PMA	48,034	42,188	39,554	44,632	58,085	47,936	51,230	55,436	50,629	5241

Data are the mean of triplicate counts for [^{3}H]thymidine uptake by 100,000 resting tonsillar B cells after culture for 2 days with CD40 mAb (1 μg/ml) or sCD40LT (1 μg/ml) in the presence of IL-4 (20 ng/ml), PMA (1n M) or CM alone. SEM < 10% of the mean values.

Table 5. Interplay between CD40 mAb and sCD40L trimer in stimulating resting B cells to DNA synthesis

	sCD40LT	BL-OGY/C4	EA5	5C3	G28-5	S2C6	HB14	M2	M3	CM
BL-OGY/C4	**26,232**[a]	2370	3619	2548	2470	4355	3922	2466	2418	2709
EA5	**50,421**	3699	5218	4183	3107	4173	3786	4952	4769	5132
5C3	**28,475**	2807	5038	2206	2649	4772	3622	6400[b]	6580	1991
G28-5	6049	2797	3419	2751	2994	2909	3833	3293	3171	2716
S2C6	**24,318**	4173	3579	4693	2606	5398	2174	4637	4552	4152
HB14	**10,243**	3339	2868	3100	3524	1757	3481	3149	3246	4106
M2	3134	1845	3404	6931	2874	4189	3401	3464	3503	3175
M3	3509	2261	3751	7562	2720	4479	3540	3774	3778	3399
CM	4073	2709	5132	1991	2716	4152	4106	3175	3399	322

Data are the mean of triplicate counts for [^{3}H]thymidine uptake by 100,000 resting tonsillar B cells after culture for 48 h with sCD40LT (1 μg/ml) and CD40 mAb (1 μg/ml) or with combinations of two CD40 mAb. SEM < 10% of the mean values.
[a]Bold and underlined values indicate synergy between agonists.
[b]Underlined values indicate additive effect of agonists.

tor following interaction with soluble trimeric CD40L would be unlikely. The inability of the ligand in this form to stimulate high-rate DNA synthesis in resting cells (Table 4 and Fig. 6) may therefore reflect limited CD40 cross-linking.

It is possible that the differing parameters necessary for influencing resting and GC B cells via CD40 relate more to the phenotypic change engendered rather than a fundamental difference in the populations *per se*. Thus, while the extracellular requirements to rescue GC B cells from apoptosis via CD40 are clearly less demanding than for the promotion of either clustering or cell cycle progression in resting B cells, the ability to sustain GC B cells in active cycle necessitates a similarly high degree of CD40 cross-linking to that noted for the resting population (Fig. 4). This is consistent with our earlier observations that transfectants expressing human CD40L can maintain proliferation of GC B cells, whereas soluble CD40 mAb cannot. Despite this, there does, however, appear to be a distinction between resting and GC B cells in the way that CD40 couples to intracellular signal transduction pathways. In GC B cells, extensive phosphorylation on tyrosine

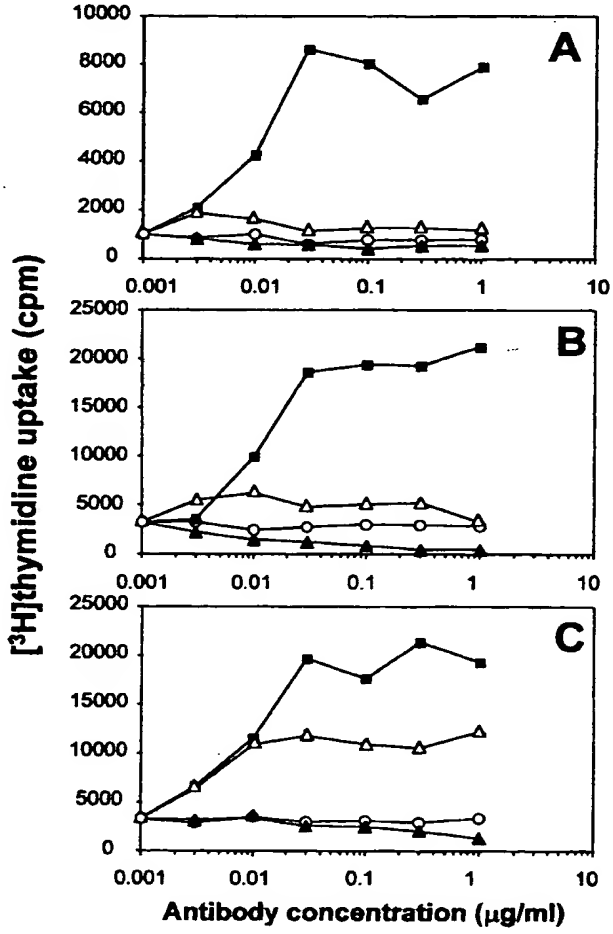


Fig. 3. Epitope-dependent cooperation between CD40 mAb and sCD40L trimer in stimulating DNA synthesis by peripheral blood B cells. Peripheral blood B cells (5×10^4/well) were cultured for 72 h with CD40 mAb and a suboptimal concentration of sCD40LT (0.5 μg/ml) after which [^{3}H]thymidine uptake was determined. (A) Cells cultured without additional co-factors; (B) in the presence of rabbit anti-IgM-Sepharose (5 μg/ml); (C) in the presence of IL-4 (5 ng/ml). ■, EA5; ▲, M2; △, S2C6; ○, mouse IgG

residues of multiple substrates has been reported (9) while for resting tonsillar B cells, either no, or limited transient, tyrosine phosphorylation has been described (9,27). It is of course possible that once GC B cells have received their initial protein tyrosine kinase-dependent survival signal via CD40, the receptor recouples to signal transduction pathways operative in the resting B cell to determine exit from, or maintenance of, cell cycle depending on the presence or absence, strength and nature, of subsequent CD40–CD40L interactions. That the requirements for rescuing group I BL cells from induced apoptosis mirrored those of suppressing spontaneous programmed death in GC B cells (Table 3) is compatible with this neoplasm being a phenotypic counterpart to the GC population and suggests that the coupling of CD40 to intracellular signal transduction pathways may be similar in these two populations.

While our studies highlight the differing extracellular requirements for promoting differential phenotypic change, it is interesting to note two recent studies detailing differential signalling through distinct cytoplasmic domains of CD40. Hostager *et al.* (28) demonstrated that a 22 amino acid truncation of residues 236–257 at the C-terminus abrogated or severely impaired the ability of CD40 to signal for increased expression of CD23, B7-1, Fas, LFA-1 and ICAM-1, while leaving B cell receptor (BCR)-dependent enhancement of CD40-stimulated antibody production intact. By contrast, an Ala subtitution of Thr234 left the pathways leading to enhanced expression of LFA-1 and ICAM-1 relatively unscathed but abrogated CD40's capacity to stimulate both the up-regulation of CD23, B7-1 and Fas, and the BCR-dependent enhancement of CD40-stimulated antibody production. It was suggested that the hCD40T^{234}A mutant may be defective through an inability to couple to the TNFR-associated factor-3 (TRAF3), while the defect in the 236–257 C-terminus deletion mutant would result from its inability to recruit TRAF2. Goldstein and Watts (29) reported that both threonine residues 227 and 234 were critical for CD40-dependent B7-1 induction but not for growth inhibition; indeed, a deletion mutant of hCD40 with only six residues remaining in the cytoplasmic tail still retained some capacity to deliver a growth inhibitory signal to transfected murine lymphoma B cells. Although there is no direct evidence at this stage, it is tempting to speculate that the way CD40 is engaged outside the cell may influence the signalling domains activated inside the cell for engendering functional change.

Another possibility that could be considered to explain the apparent differing requirements for signalling GC and resting B cells via CD40 is that the former but not the latter express a CD40L. This could be the already-characterized T cell-associated CD40L (30,31) or a novel B cell-associated counter-structure (15). It is interesting to note that, in all three studies, expression of a CD40L was restricted to activated B cells. If GC B cells—or at least a subset of them—were to express a functional CD40 counter-structure, then this could potentially cooperate with any added CD40 signal to engender phenotypic change.

The finding that mAb binding to distinct epitopes of CD40 can function differently (Tables 3 and 4) demonstrates that there may be an allosteric component to some aspects of CD40 signalling. Although this would be consistent with, it by no means demonstrates, the existence of a second CD40 counter-structure. One possibility to account for the differential behaviour of the CD40 mAb is that they differ in their ability to promote, or inhibit, CD40 microaggregates at the B cell surface. The relative spatial relationships between epitopes defined by the mAb studied and the CD40L binding site appears to be as shown in Fig. 7. This assignment is based on the following information from the present study: (i) competition of CD40L binding (Fig. 2 and Table 2), (ii) competitive binding with CD40 mAb S2C6 (Table 1), and (iii) interplay between mAb with each other and with sCD40LT for promotion of DNA synthesis in resting B cells (Table 5).

CD40 mAb EA5 and 5C3, which could partially inhibit equilibrium binding of sCD40LT to immobilized CD40–Fc (Table 2), apparently also accelerated receptor–ligand exchange under the same conditions (Table 2). The likely

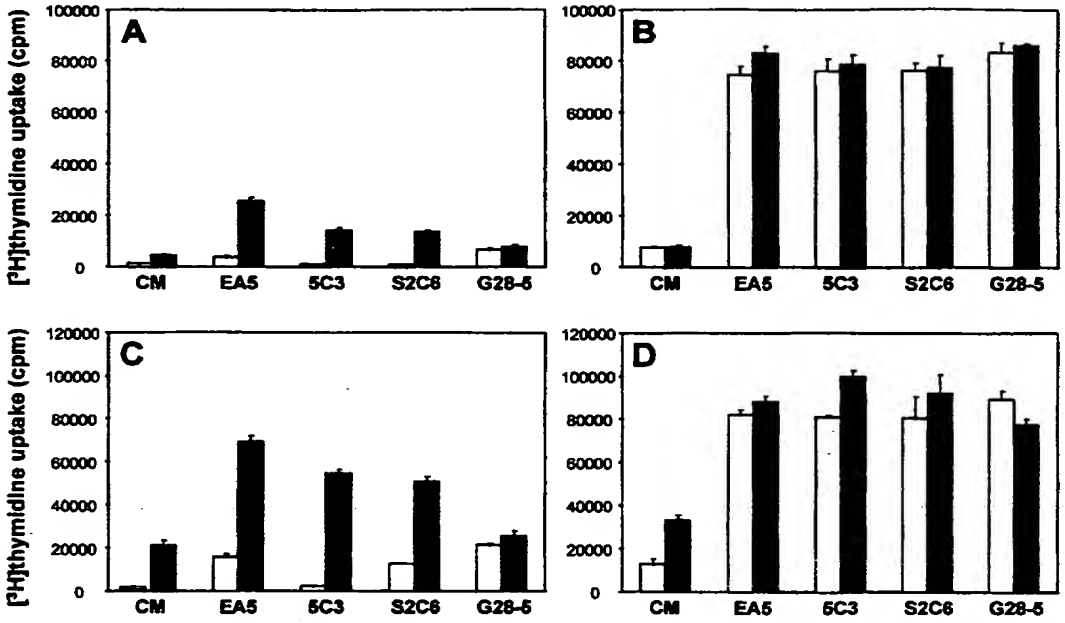


Fig. 4. Stimulation of DNA synthesis in tonsillar B cell subsets by CD40 mAb and sCD40L trimer: influence of co-culture with CD32 transfectants. Tonsillar resting B cells (A and B) or GC B cells (C and D) (100,000 cells/well) were cultured with CD40 mAb (1 μg/ml) in the presence (closed bars) or absence (open bars) of sCD40LT (1/5 dilution of CD40LT-transfected COS cell supernatant). (B and D) Responses of cells cultured with CD32-L cell transfectants (10,000/well). [^{3}H]Thymidine uptake was determined after 48 h. Data are means ± SEM for triplicate measurements. Responses to stimulation with PMA (1 nM) and ionomycin (0.8 μg/ml) were 80,000–90,000 c.p.m. for resting B cells and 90,000–100,000 c.p.m. for GC B cells.

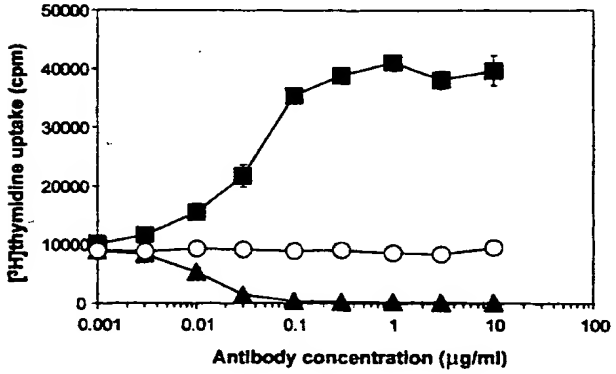


Fig. 5. Stimulation of DNA synthesis in peripheral blood B cells by trimeric sCD40L after cross-linking by antibody. Peripheral blood B cells (5×10^4/well) were cultured for 72 h with sCD40LT (500 ng/ml) in the presence of CD40L mAb M79 (■), M91 (▲) or mouse IgG (○) after which time [^{3}H]thymidine uptake was determined. M91 blocks CD40L binding while M79 does not (18).

mechanism for this is that the ability of the trivalent ligand to stably cross-link this bivalent form of the receptor is altered when these mAb are bound. Enhanced binding of CD40–Fc to the CD40L expressed on T cells after pre-incubation with 5C3 (Table 1) may reflect the ability of the antibody to promote further cross-linking of receptor–ligand complexes at the cell surface by interacting with epitopes outside the ligand binding site.

In an earlier study, Bjork *et al.* (32) also found that antibody S2C6 was able to partially block CD40L binding and noted that—in the presence of IL-4—S2C6 showed cooperation with sCD40LT for stimulating both DNA synthesis and IgE production in human B cells. Using a different set of mAb to the ones investigated in detail in our study, Lindhout *et al.* (33) found that whilst they were poor at stimulating resting B cells into proliferation, they were capable of suppressing apoptosis in the 'light density' B cell fraction from tonsils: these would have included GC B cells and is thus consistent with the notion that there are epitope differences in terms of the function engendered through CD40. Probably due to the fewer number of antibodies to CD40 available, there is less information on epitope distribution and function in mouse but Heath *et al.* (14) have reported on two mAb which appear to bind to different sites on murine CD40 and exhibit differential behaviour.

Teleologically, it is not readily apparent as to why the requirements for rescuing GC B cells via CD40 should be substantially less rigorous than for stimulating B cells into and through cycle. It may possibly relate to the relatively low (but not insignificant) number of T cells available at the presumed physiological site of CD40-dependent rescue of antigen-selected centrocytes in the GC light zone (34) such that if the demands were too high then useful V gene mutations could be lost through insufficient signalling. This would need

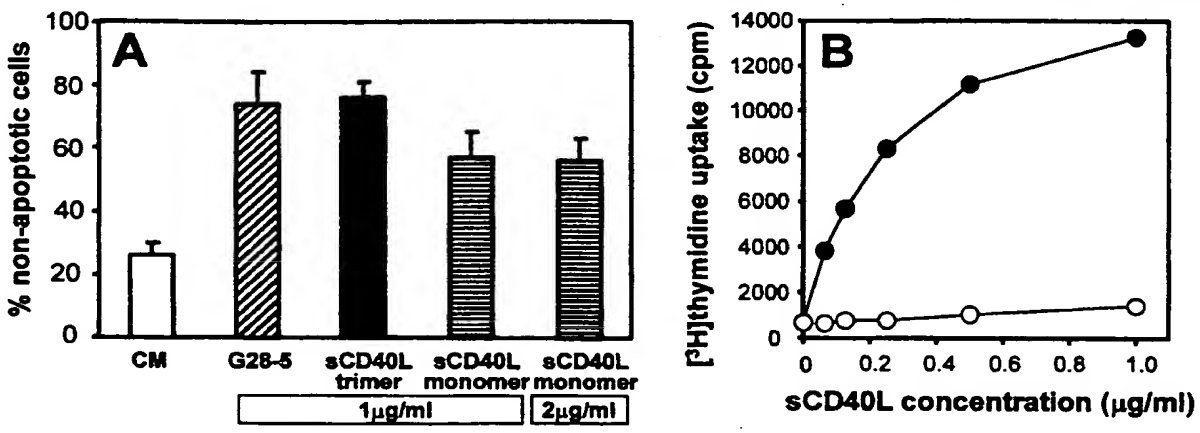


Fig. 6. Monomeric sCD40L can rescue GC B cells from apoptosis but cannot stimulate resting B cells into cell cycle. (A) Tonsillar GC B cells (10^6/ml) were cultured with CM alone, CD40 mAb G28-5 (1 μg/ml), sCD40L trimer (1 μg/ml) or sCD40L monomer (1 or 2 μg/ml). The percentage of non-apoptotic cells remaining was determined after 24 h. Data are means ± SD for three experiments. (B) Tonsillar resting B cells (100,000/well) were cultured with sCD40L trimer (●) or monomer (○) at various concentrations and [^{3}H]thymidine uptake was determined after 48 h.

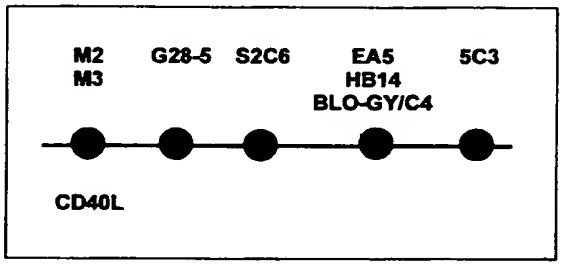


Fig. 7. Spatial relationship between epitopes on CD40 suggested by this study.

to be balanced with the possibility that any naturally produced soluble CD40L in the GC could rescue worthless mutations in a non-cognate fashion. Perhaps the rare escape of an autoreactive mutation as evidenced in rheumatoid arthritis or systemic lupus erythematosus might be accounted for by occasional noise in such fine tuning. It is of interest that continued proliferation—and, thus, possibly the rerouting of centrocytes back to the dark zone to accumulate further mutations in antigen receptor—requires prolonged exposure to cell bound CD40L, suggesting a mechanism for minimizing the production of particularly high-affinity somatic mutations to self.

Soluble CD40L [synonym, TNF-related activation protein (TRAP)] has been reported to be released by activated T_h cells *in vitro* (35) indicating at least the possibility of the above scenario arising *in vivo*. This material, characterized as an 18 kDa monomer, did not result from cleavage of cell surface CD40L but may have been produced by processing within an intracellular compartment (35). Naturally produced soluble CD40L has subsequently been shown to down-regulate CD40 on dendritic cells and to produce a long-lasting anti-apoptotic effect (36). While the monomeric recombinant CD40L used in our study was able to rescue GC B cells from apoptosis we wish to stress that this does not necessarily imply that univalent tethering of CD40 is sufficient for such rescue. Although monomeric at the concentration maintained in solution, it is possible that once bound to the cell surface a degree of spontaneous aggregation occurs sufficient to engender a functional signal. The only conclusion we can, or wish to, reach is that the experiments using 'monomeric' CD40L further highlight that the requirements for suppressing apoptosis and inducing proliferation via CD40 are less rigid for the former than the latter. We are currently exploring the intracellular signal transduction mechanisms that may underlie not only this but also the differences observed in CD40 mAb to promote homotypic adhesions.

Acknowledgements

This work was funded in part by program grants from the Medical Research Council (UK) and the EU (BIO2CT-CT920269 and BIO4-CT95-0252), and by a project grant from the Leukaemia Research Fund. J. G. is an MRC Professor.

Abbreviations

AET	aminoethylisothiouronium bromide
BCR	B cell (antigen) receptor
BL	Burkitt's lymphoma
CD40L	CD40 ligand
CM	culture medium
GaHIg	goat anti-human IgG1
GC	germinal center
NGFR	nerve growth factor receptor
PBMC	peripheral blood mononuclear cells
PE	phycoerythrin
PMA	phorbol myristate acetate
sCD40L	soluble CD40 ligand
sCD40LT	soluble CD40 ligand trimer
SPR	surface plasmon resonance
SRBC	sheep red blood cell
TNFR	tumour necrosis factor receptor
TRAF	TNFR-associated factor

References

1 Wheeler, K., Pound, J. D., Gordon, J. and Jefferis, R. 1993. Engagement of CD40 lowers the threshold for activation of resting B cells via antigen receptor. *Eur. J. Immunol.* 23:1165.

2 Barrett, T. B., Shu, G. and Clark, E. A. 1991. CD40 signaling activates CD11a/CD18 (LFA-1)-mediated adhesion in B cells. *J. Immunol.* 146:1722.

3 Flores-Romo, L., Estoppey, D. and Bacon, K. B. 1993. Anti-CD40 antibody stimulates the VLA-4-dependent adhesion of normal and LFA-1-deficient B cells to endothelium. *Immunology* 79:445.

4 Cairns, J. A., Flores-Romo, L., Millsum, M. J., Guy, G. R., Gillis, S., Ledbetter, J. A. and Gordon, J. 1988. Soluble CD23 is released by B lymphocytes cycling in response to interleukin 4 and anti-Bp50 (CDw40). *Eur. J. Immunol.* 18:349.

5 Armitage, R. J., Fanslow, W. C., Strockbine, L. , Sato, T. A., Clifford, K. N., MacDuff, B. M., Anderson, D. M., Gimpel, S. D., Davis-Smith, T., Maliszewski, C. R., Clark, E. A., Smith, C. A., Grabstein, K. H., Cosman, D. and Spriggs, M. K. 1992. Molecular and biological characterization of a murine ligand for CD40. *Nature* 357:80.

6 MacLennan, I. C. M., Gulbranson-Judge, A., Toellner, K. M., Casamayor-Palleja, M., Chan, E. Y. T., Sze, D. M.-Y., Luther, S. A. and Orbea, H. A. 1997. The changing preference of T and B cells for partners as T-dependent antibody responses develop. *Immunol. Rev.* 156:53.

7 Gregory, C. D., Dive, C., Henderson, S., Smith, C. A., Williams, G. T. Gordon, J. and Rickinson, A. B. 1991. Activation of Epstein–Barr virus latent genes protects human B cells from death by apoptosis. *Nature* 349:612.

8 Gordon, J. 1995. CD40 and its ligand: central players in B lymphocyte survival, growth and differentiation. *Blood* 9:53.

9 Knox, K. A. and Gordon, J. 1993. Protein tyrosine phosphorylation is mandatory for CD40-mediated rescue of germinal center B cells from apoptosis. *Eur. J. Immunol.* 23:2578.

10 Holder, M. J., Wang, H., Milner, A. E., Casamayor-Palleja, M., Armitage, R. J., Spriggs, M. K., Fanslow, W. C., MacLennan, I. C. M., Gregory, C. D. and Gordon, J. 1993. Suppression of apoptosis in normal and neoplastic human B lymphocytes by CD40 ligand is independent of Bcl-2 induction. *Eur. J. Immunol.* 23:2368.

11 Mallett, S. and Barclay, A. N. 1991. A new superfamily of cell surface proteins related to the nerve growth factor receptor. *Immunol. Today* 12:220.

12 Gruss, H. J. and Dower, S. K. 1995. Tumor necrosis factor ligand superfamily: involvement in the pathology of malignant lymphomas. *Blood* 85:3378.

12 Bjorck, P. and Paulie, S. 1996. CD40 antibodies defining distinct epitopes display qualitative differences in their induction of B-cell differentiation. *Immunology* 87:291.

14 Heath, A. W., Wu, W. W. and Howard, M. C. 1994. Monoclonal antibodies to murine CD40 define two distinct functional epitopes. *Eur. J. Immunol.* 24:1828.

15 Howard, M. C., Heath, A. W., Ishida, H. and Moore, K. W. 1992. Biological roles of IL-10 and the CD40 receptor. *Progr. Immunol.* VIII:327.

16 Katira, A., Holder, M. J., Pound, J. D. and Gordon, J. 1995. CD40 Workshop Panel Report. In Schlossman, S. F., Boumsell, L., Gilks, W., Harlan, J. M., Kishimoto, T., Morimoto, C., Ritz, J. , Shaw, S., Silverstein, R., Springer, T., Tedder, T. F. and Todd, R. F., eds, *Leukocyte Typing V*, p. 547. Oxford University Press, Oxford.

17 Fanslow, W. C., Clifford, K. N., Zappone, J., Alderson, M. R. and Armitage, R. J. 1995. CD40 mAb M2 and M3 inhibit CD40 ligand binding and function. In Schlossman, S. F., Boumsell, L., Gilks, W., Harlan, J. M., Kishimoto, T., Morimoto, C., Ritz, J. , Shaw, S., Silverstein, R., Springer, T., Tedder, T. F. and Todd, R. F., eds, *Leukocyte Typing V*, p. 555. Oxford University Press, Oxford.

18 Fanslow, W. C., Rousseau, A.- M. C., Lofton, T. E., Klinke, R., Ulrich, D. T. and Armitage, R. J. 1998. CD154 (CD40 ligand) workshop panel report. In Kishimoto, T., Kikutani, H., von dem Borne, A. E. G. K., Goyert, S. M., Mason, D. Y., Miyasaka, M., Moretta, L., Okumura, K., Shaw, S., Springer, T. A., Sugamura, K. and Zola, H., eds, *Leucocyte Typing VI. White Cell Differentiation Antigens*, p. 101. Garland, New York.

19 Fanslow, W. C., Srinivasan, S., Paxton, R., Gibson, M. G., Spriggs, M. K. and Armitage, R. J. 1994. Structural characteristics of CD40 ligand that determine biological function. *Semin. Immunol.* 6:267.

20 Fanslow, W. C., Anderson, D. M., Grabstein, K. H., Clark, E. A., Cosman, D. and Armitage, R. J. 1992. Soluble forms of CD40 inhibit biologic responses of human B cells. *J. Immunol.* 149:655.

21 Pound, J. D. and Gordon, J. 1997. Maintenance of human germinal center B cells *in vitro*. *Blood* 89:919.

22 Arend, W. P., Malyak, M., Smith, M. F., Jr, Whisenand, T. D. , Slack, J. L., Sims, J. E., Giri, J. G. and Dower, S. K. 1994. Binding of IL-1α, IL-1β, and IL-1 receptor antagonist by soluble IL-1 receptors and levels of soluble IL-1 receptors in synovial fluids. *J. Immunol.* 153:4766.

23 Dower, S. K., Titus, J. A., DeLisi, C. and Segal, D. M. 1981. Mechanism of binding of multivalent immune complexes to Fc receptors. 2. Kinetics of binding. *Biochemistry* 20:6335.

24 Liu, Y.- J., Cairns, J. A., Holder, M. J., Abbot, S. D., Jansen, K. U., Bonnefoy, J.-Y., Gordon, J. and MacLennan, I. C. M. 1991. Recombinant 25-kDa CD23 and interleukin 1a promote the survival of germinal center B cells: evidence for bifurcation in the development of centrocytes rescued from apoptosis. *Eur. J. Immunol.* 21:1107.

25 MacDonald, I., Wang, H., Grand, R., Armitage, R. J., Fanslow, W. C., Gregory, C. D. and Gordon, J. 1996. Transforming growth factor-1 cooperates with anti-immunoglobulin for the induction of apoptosis in group I (biopsy-like) Burkitt lymphoma cell lines. *Blood* 87:1147.

26 Bajorath, J., Marken, J. S., Chalupny, N. J. , Spoon, T. L., Siadak, A. W., Gordon, M., Noelle, R. J., Hollenbaugh, D. and Aruffo, A. 1995. Analysis of gp39/CD40 interactions using molecular models and site-directed mutagenesis. *Biochemistry* 34:9884.

27 Uckun, F. M., Schieven, G. L., Dibirdik, I. , Chandan-Langlie, M., Tuel-Ahlgren, L. and Ledbetter, J. A. 1991. Stimulation of protein tyrosine phosphorylation, phosphoinositide turnover, and multiple previously unidentified serine/threonine-specific protein kinases by the pan-B-cell receptor CD40/Bp50 at discrete developmental stages of human B-cell ontogeny. *J. Biol. Chem.* 266:17478.

28 Hostager, B. S., Hsing, Y., Harms, D. E. and Bishop, G. A. 1996. Different CD40-mediated signaling events require distinct CD40 structural features. *J. Immunol.* 157:1047.

29 Goldstein, M. D. and Watts, T. H. 1996. Identification of distinct domains in CD40 involved in B7-1 induction or growth inhibition. *J. Immunol.* 157:2837.

30 Grammer, A. C., Bergman, M. C., Miura, Y., Fujita, K., Davis, L. S. and Lipsky, P. E. 1995. The CD40 ligand expressed by human B cells costimulates B cell responses. *J. Immunol.* 154:4996.

31 Wykes, M., Poudrier, J., Lindstedt, R. and Gray, D. 1998. Regulation of cytoplasmic, surface and soluble forms of CD40 ligand in mouse B cells. *Eur. J. Immunol* 28:548.

32 Bjorck, P., Braesch-Andersen, S. and Paulie, S. 1994. Antibodies to distinct epitopes on the CD40 molecule co-operate in stimulation and can be used for the detection of soluble CD40. *Immunology* 83:430.

33 Lindhout, E., Lakeman, A. and de Groot, C. 1995. Follicular dendritic cells inhibit apoptosis in human B lymphocytes by a rapid and irreversible blockade of pre-existing endonuclease. *J. Exp. Med.* 181:1985.

34 Casamayor-Palleja, M., Khan, M. and MacLennan, I. C. M. 1995. A subset of $CD4^+$ memory T cells contains preformed CD40 ligand that is rapidly but transiently expressed on their surface after activation through the T cell receptor complex. *J. Exp. Med.* 181:1293.

35 Graf, D., Muller, S., Korthauer, U., van Kooten, C., Weise, C. and Kroczek, R. A. 1995. A soluble form of TRAP (CD40 ligand) is rapidly released after T cell activation. *Eur. J. Immunol.* 25:1749.

36 Ludewig, B., Henn, V., Schroder, J. M., Graf, D. and Kroczek, R. A. 1996. Induction, regulation, and function of soluble TRAP (CD40 ligand) during interaction of primary $CD4^+$ $CD45RA^+$ T cells with dendritic cells. *Eur. J. Immunol.* 26:3137.

29. **Bjorkman, P. J., Saper, M. A., Samraoui, B., Bennett, W. S., Strominger J. L., and Wiley, D. C.**, The foreign antigen binding site and T cell recognition regions of class I histocompatibility antigens, *Nature*, 329, 506, 1987.
30. **Stern, L. J., Brown, J. H., Jardetzky, T. S., Gorga, J. C., Urban, R. G., Strominger, J. L., and Wiley, D. C.**, Crystal structure of the human class II MHC protein HLA-DR1 complexed with an influenza virus peptide, *Nature*, 368, 215, 1994.
31. **Catipovic, B., Dalporto, J., Mage, M., Johansen T. E., and Schneck, J. P.**, Major histocompatibility complex conformational epitopes are peptide specific, *J. Exp. Med.*, 176, 1611, 1992.
32. **Bluestone, J. A., Jameson, S., Miller, S., and Dick, R.**, Peptide-induced conformational changes in class I heavy chains alter major histocompatibility complex recognition, *J. Exp. Med.*, 176, 1757, 1992.
33. **Fremont, D. H., Matsumura, M., Stura, E. A., Peterson, P. A., and Wilson, I. A.**, Crystal structures of two viral peptides in complex with murine MHC class I H-2K^b, *Science*, 257, 919, 1992.
34. **Madden, D. R., Garboczi, D. N., and Wiley, D. C.**, The antigenic identity of peptide-MHC complexes: a comparison of the conformations of five viral peptides presented by HLA-A2, *Cell*, 75, 693, 1993.
35. **Ehrich, E. W., Devaux, B., Rock, E. P., Jorgensen, J. L., Davis, M. M., and Chien, Y. H.**, T cell receptor interaction with peptide/major histocompatibility complex (MHC) and superantigen/MHC ligands is dominated by antigen, *J. Exp. Med.*, 178, 713, 1993.
36. **Chien, Y. H. and Davis, M. M.**, How alpha-beta T-cell receptors 'see' peptide/MHC complexes, *Immunol. Today*, 14, 597, 1993.
37. **Sant'Angelo, D. B., Waterbury, G., Preston-Hurlburt, P., Yoon, S. T., Medzhitov, R., Hong, S. C., and Janeway, C. J.**, The specificity and orientation of a TCR to its peptide-MHC class II ligands, *Immunity*, 4, 367, 1996.
38. **Janeway, C. A. and Bottomly, K.**, Responses of T cells to ligands for the T-cell receptor, *Semin. Immunol.*, 8, 109, 1996.
39. **Garcia, K. C., Degano, M., Stanfield, R. L., Brunmark, A., Jackson, M. R., Peterson, P. A., Teyton, L., and Wilson, I. A.**, An ab T cell receptor at 2.5 Å and its orientation in the TCR-MHC complex, *Science*, 274, 209, 1996.
40. **Garboczi, D. N., Ghosh, P., Utz, U., Fan, Q. R., Biddison, W. E., and Wiley, D. C.**, Structure of the complex between human T cell receptor, viral peptide, and HLA-A2, *Nature*, 384, 134, 1996.
41. **Brock, R., Weismuller, K.-H., Jung, G., and Walden, P.**, Molecular basis for the recognition of two structurally different major histocompatibility complex/peptide complexes by a single T-cell receptor, *Proc. Natl. Acad. Sci. U.S.A.*, 93, 13108, 1996.
42. **Herman, A., Parham, P., Weissman, S. M., and Engelhard, V. H.**, Recognition by xenogeneic cytotoxic T lymphocytes of cells expressing HLA-A2 or HLA-B7 after DNA-mediated gene transfer, *Proc. Natl. Acad. Sci. U.S.A.*, 80, 5056, 1983.
43. **Henderson, R. A., Cox, A. L., Sakaguchi, K., Appella, E., Shabanowitz, J., Hunt, D. F., and Engelhard, V. H.**, Direct identification of an endogenous peptide recognized by multiple HLA-A2.1-specific cytotoxic T cells, *Proc. Natl. Acad. Sci. U.S.A.*, 90, 10275, 1993.
44. **Sherman, L. A. and Chattopadhyay, S.**, The molecular basis of allorecognition, *Annu. Rev. Immunol.*, 11, 385, 1993.
45. **Udaka, K., Tsomides, T. J., and Eisen, H. N.**, A naturally occurring peptide recognized by alloreactive CD8$^+$ cytotoxic T lymphocytes in association with a class I MHC protein, *Cell*, 69, 989, 1992.

Critical Reviews™ in Immunology, 17:427–435 (1997)

Agonistic Activity of a CD40-Specific Single-Chain Fv Constructed from the Variable Regions of mAb G28-5

Jeffrey A. Ledbetter, Joseph A. Francisco, Clay B. Siegall, Lisa K. Gilliland, Diane Hollenbaugh, Alejandro Aruffo, Anthony W. Siadak, Nicole Mischel-Petty, Laura S. Grosmaire, Marcia L. Gordon, T. Joseph Brown, Patti Moran-Davis, Robert S. Mittler, Peter A. Kiener, and Steven G. Nadler**

Bristol-Myers Squibb Pharmaceutical Research Institute, 3005 First Avenue, Seattle, WA 98121

* Authors to whom all correspondence should be addressed.

ABSTRACT: A single-chain Fv (sFv) was expressed from the variable regions of the CD40-specific mAb G28-5. The molecule bound CD40 with a high affinity (2.2 n*M*) and was a monomer in solution. Surprisingly, G28-5 sFv was a potent CD40 agonist that rapidly crosslinked CD40 on the cell surface but did not crosslink CD40-Ig in solution. G28-5 sFv was a more potent agonist than G28-5 IgG and was able to stimulate CD40 responses by B cells and monocytes. G28-5 IgG partially blocked, whereas G28-5 sFv augmented CD40 responses during stimulation with natural ligand (gp39-CD8 fusion protein). These results indicate that the functional activity of ligands built from the binding site of G28-5 is highly dependent upon the size and physical properties of the molecule both in solution and on the cell surface.

KEY WORDS: CD40, sFv, antibody crosslinking, NF-κB.

I. INTRODUCTION

The function of the CD40 receptor has been studied for many years using monoclonal antibody (mAb) G28-5 to stimulate a CD40 response.[1–3] Crosslinking of CD40 has been identified as an important requirement for signal transduction,[4,5] a feature shared by other members of the CD40 family, including TNFR, FAS, and others.[6–8] A natural trimeric ligand for CD40 (CD154, formerly gp39 or CD40L) that is a TNF-α family member and is expressed by helper T cells was discovered more recently.[9,10] This molecule has been produced as a soluble fusion protein with the extracellular domain of murine CD8 (gp39-MCD8)[11] to allow a direct comparison with CD40 mAb for the ability to stimulate responses in B cells, monocytes, and endothelial cells. The comparison showed that G28-5 is not a full agonist for the CD40 receptor, but is a partial agonist/partial antagonist that stimulates but also inhibits full activation from CD154.[4] A monovalent Fab fragment generated by papain digestion of G28-5 did not directly stimulate and was primarily antagonistic for CD40 responses to natural CD40 ligand.[4] These results led us to construct a monovalent single-chain Fv (sFv) from the variable regions of G28-5 in an attempt to generate a CD40 inhibitor that could be expressed without the need for proteolytic cleavage.

Here we present data showing that G28-5 sFv is a more potent agonist for CD40 responses by B cells and monocytes than the parental G28-5 IgG, and that the sFv form of the molecule does not retain any of the antagonistic properties of the IgG or its Fab fragment. However, the sFv was not identical to natural ligand because gp39-CD8 could induce expression of adhesion molecules

1040-8401/97/$5.00

by endothelial cells, whereas the sFv could not. Consistent with the agonistic activity, G28-5 sFv rapidly induced CD40 clustering on B cells, even though analysis by HPLC showed that the sFv was a 16-kDa monomer in solution. These results suggest that CD40 ligands constructed from mAb G28-5 variable regions can selectively stimulate CD40 responses in a cell lineage-dependent fashion that differs from the activity profile of natural CD40 ligand.

A. Construction, Expression, and Purification of G28-5 sFv

Cloning of the G28-5 variable regions and construction of G28-5 sFv in the V_L-V_H orientation, fused to a binding defective truncated form of *Pseudomonas* exotoxin (PE40), encoded by pSE51, have been previously described.[12] For expression of G28-5 sFv, the sFv domain of pSE51 was PCR amplified to include a stop codon following the V_H region. pSE51 was subsequently digested with EcoR1 and NdeI to remove the coding region of G28-5 sFv-PE40 and the PCR amplified G28-5 sFv was ligated into the vector following digestion with the same restriction enzymes. Protein expression and purification of the resulting plasmid pG28-5 sFv was essentially as previously described.[12] Briefly, *E. coli* strain BL21(λDE3) was transformed with pG28-5 sFv and cultured at 37°C in Superbroth (Digene, Silver Spring, MD) containing 50 μg/ml ampicillin. When the culture reached $OD_{600} = 1.0$, expression of the sFv was induced for 90 min with 1 m*M* isopropyl β-D-thiogalactoside. The cells were harvested by centrifugation and inclusion bodies were isolated as previously described.[13] After solubilization of the inclusion bodies in 7 *M* guanidine-HCl/100 m*M* Tris-HCl (pH 7.4)/5 m*M* EDTA, the sFv was refolded at 50 μg/ml in 100 m*M* Tris-HCl (pH 8.0)/5 m*M* EDTA/1 *M* urea/1 m*M* reduced glutathione/0.1 m*M* oxidized glutathione. The refolded sFv was purified through a two-step procedure, by affin-ity chromatography, utilizing immobilized CD40-Ig and then size exclusion chromatography to remove aggregates. The purified sFv was a single peak of 16kDa by HPLC gel filtration analysis, and migrated as a single 27kDa band on SDS gels with or without reduction (data not shown).

B. CD40 Binding by G28-5 sFv

The binding of G28-5 sFv to CD40 on the Ramos B-cell line was examined by measuring the ability of the sFv to block binding of parental G28-5 IgG. These experiments show that the sFv completely blocked the binding of G28-5 IgG in a dose-dependent manner. Thus, G28-5 sFv binds specifically to CD40 with a binding affinity sufficient to block the binding of G28-5 IgG. To determine the affinity of binding, G28-5 sFv, CD40 was directly labeled with ^{125}I and binding assays with CD40 positive B-cell lines were performed. G28-5 sFv showed saturable binding and a binding affinity of 2.2 n*M* (data not shown).

C. Agonistic Activity of G28-5 sFv for B-Cell CD40 Responses

The functional effect of G28-5 sFv on CD40 responses was examined. Dense B cells isolated from tonsils were stimulated with various CD40 ligands alone or together and proliferation was measured by uptake of ^3H-thymidine. G28-5 sFv but not G28-5 IgG directly induced proliferation, and activity was detectable at concentrations as low as 10 ng/ml (0.4 n*M*). A combination of the sFv with gp39-mCD8 gave rise to a specific dose-dependent synergy in the stimulation of proliferation (Figure 1). In contrast, G28-5 IgG inhibited the activity of the gp39-mCD8 molecule. These results show that the activity of G28-5 sFv is distinct from that of the parental IgG.

We also examined the effects of G28-5 sFv on B-cell proliferation when given as a costimulatory signal with $F(ab')_2$ anti-IgM, PMA, IL4, or CD20 mAb 1F5. B-cell proliferation induced by anti-IgM was markedly enhanced by G28-5 sFv, whereas G28-5 IgG had much less effect at all concentrations of anti-IgM or CD40 ligand (Figures 1B and C). Similarly, the sFv was a more potent agonist than G28-5 IgG in costimulation assays with anti-CD20 or with PMA (Table 1). Furthermore, the sFv did not inhibit activity of gp39-mCD8, whereas Fab fragments of mAb G28-

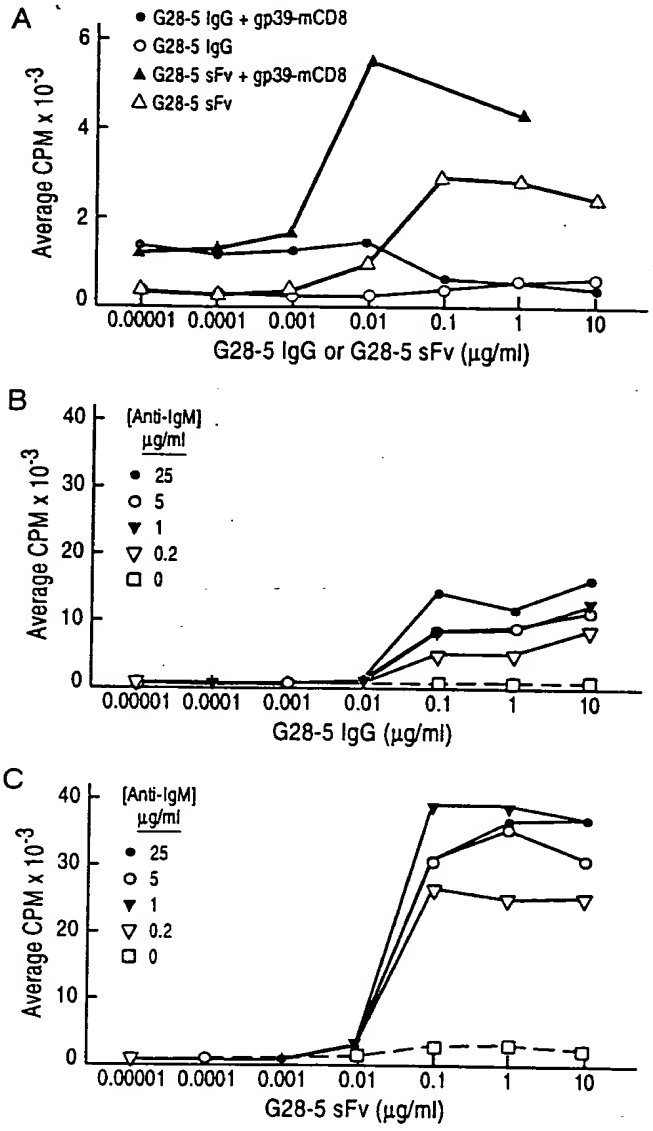


FIGURE 1. Effect of G28-5 sFv on B-cell proliferation. Resting B cells were isolated from tonsils by E-rosetting to remove T cells followed by separation on Percoll to collect dense cells as described.[24] (A) Direct stimulation of B-cell proliferation with CD40 ligands, using G28-5 IgG or G28-5 sFv at the indicated concentrations, and gp39-mCD8 as a 1:4 dilution of supernatant from transfected COS cells. Proliferation induced by gp39-mCD8 alone was 1371 +/– 206 cpm. (B) Costimulation of B-cell proliferation by G28-5 IgG using varying amounts of anti-IgM immunobeads (Irvine Scientific, Santa Ana, CA) as indicated. (C) Costimulation of B-cell proliferation by G28-5 sFv using the same concentration range of anti-IgM immunobeads as indicated.

5 did inhibit the gp39-mCD8 costimulation with anti-CD20 (Table 1). These results indicate that G28-5 sFv is a full agonist on B cells, whereas the parental IgG or Fab fragments of G28-5 are partial agonists but are also partial antagonists for CD40 responses to natural ligand.

Consistent with its ability to induce B-cell proliferation, the G28-5 sFv also directly induced homotypic adhesion in B-cell lines at concentrations as low as 10 ng/ml, and was more potent than either gp39-mCD8 or G28-5 IgG in this assay. The size of the cell aggregates induced by G28-5 sFv was larger than those induced by other CD40 agonists, including the gp39-mCD8 fusion protein (data not shown).

D. Activity of G28-5 sFv on Monocytes and Endothelial Cells

CD40 stimulation with gp39-mCD8 directly induces functional responses in monocytes, including upregulation of adhesion molecules, prevention of apoptosis after serum withdrawal, and release of proinflamatory cytokines.[14,15] We therefore examined CD40 functional responses of elutriated monocytes to compare the effects of CD40 stimulation using gp39-mCD8 versus G28-5 sFv. The sFv-induced homotypic aggregation of purified blood monocytes at concentrations down to 50 ng/ml and was equally effective as

TABLE 1
Effect of G28-5 sFv on Tonsil B-Cell Proliferation[a]

Mean proliferation (^{3}H-thymidine incorporation, cpm × 10^{-3})

CD40 Stimulation[b]	Costimulation		
	None	CD20 (1F5, 1μg/ml)	PMA (1 ng/ml)
None	0.1	0.3	2.3
G28-5 sFv	1.2	39.0	43.7
gp39-mCD8	3.0	34.6	11.4
G28-5 Fab	0.3	2.8	19.7
G28-5	0.2	3.1	27.4
G28-5 sFv + gp39-mCD8	2.2	43.7	67.6
G28-5 Fab +gp39-mCD8	0.6	8.4	40.8
G28-5 sFv +G28-5	0.6	12.3	37.8

[a] Proliferation of dense tonsil B cells was measured by uptake of ^{3}H-thymidine during the last 16 hours of a 5-day culture. Determinations were done in quadruplicate, and standard errors were less than 15% of the mean at each point.

[b] CD40 stimulation was done using G28-5 sFv at 2 μg/ml, gp39-mCD8 at a 1/16 dilution of supernatant from transfected COS cells, G28-5 at 1 μg/ml.

gp39-mCD8 in both the size of the induced cellular aggregates and the concentration of agonist required for a detectable response (data not shown). We also found that the sFv was active in stimulation of TNF-α production, but was a weaker agonist than gp39-mCD8 and required 5 to 10 (μg/ml for activity (Figure 2A). The sFv was also able to prevent apoptosis induced by serum deprivation, and was similar to gp39-mCD8 in requiring 5 (μg/ml for maximal activity (Figure 2B). Direct induction of proliferation of purified monocytes was observed after CD40 stimulation, and gp39-mCD8 was significantly more potent than the sFv. However, in the presence of the sFv and gp39-mCD8 together, the monocyte proliferation response was enhanced (data not shown). These results show that the sFv was active in all of the monocyte assays tested, but higher concentrations of sFv were required to stimulate monocyte CD40 responses than were required to stimulate B-cell CD40 responses.

CD40 is known to be expressed and functionally active in primary endothelial cell cultures.[16,17] Gp39-mCD8 induced endothelial cell expression of adhesion molecules E-selectin and ICAM-1 and stimulated adhesion of neutrophils to the endothelial cell monolayer within 4 to 6 hrs. We found that the G28-5 sFv was unable to induce expression of E-selectin on endothelial cells even at high concentrations (5 (μg/ml) (Figure 3). Additionally, the sFv at concentrations below 50 ng/ml enhanced the activity of gp39-mCD8, whereas G28-5 IgG inhibited (data not shown). Furthermore, G28-5 sFv was able to relieve the blocking of gp39-mCD8 activity by G28-5 IgG (Figure 3), reflecting a difference in the antagonistic properties of the sFv versus IgG.

E. CD40 Clustering and Activation of NF-κB

To determine the effects of G28-5 sFv on CD40 clustering on viable cells, Raji cells were incubated for 2 hrs at 37°C with G28-5 IgG, sFv, or Fab fragments, followed by staining at 4°C for CD40 distribution using a CD40 mAb (1.87) that is not blocked by G28-5. The cells were then

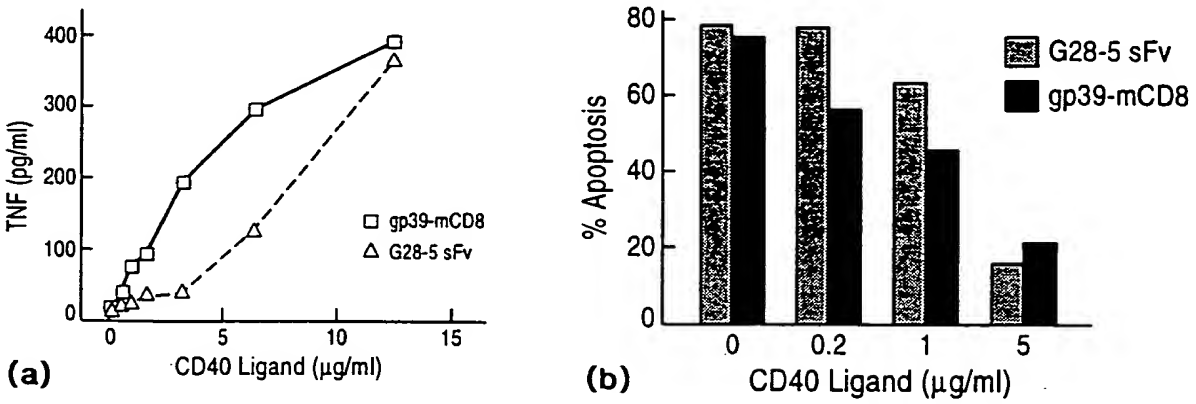


FIGURE 2. G28-5 sFv functional effects on purified monocytes. Monocytes were prepared from healthy donors by separation of the peripheral blood mononuclear cells (PBMC) on ficoll. The T cells were depleted from this fraction by rosetting with sheep red blood cells and the monocytes were separated from the remaining PBMC by elutriation. The isolation of the monocytes was carried out in RPMI containing 2.5 m*M* EDTA and 10 μg/ml polymyxin B. (A) TNFα levels in supernatants was measured by ELISA (Biosource International, Camarillo, CA) after 5 hrs of stimulation with immobilized CD40 ligands at the indicated concentrations. (B) Apoptosis of monocytes induced by serum deprivation was determined after 24 hrs by analysis of DNA content with propidium iodide staining of permeabilized cells. CD40 stimulation was with gp39-mCD8 or G28-5 sFv added in solution at the indicated concentrations.

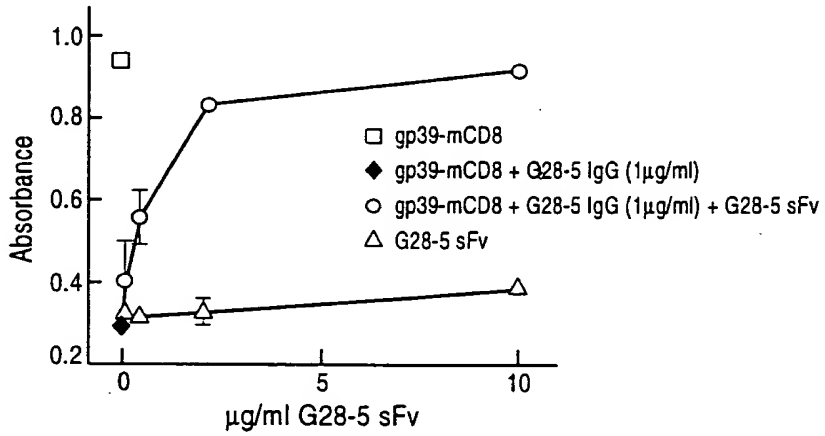


FIGURE 3. Stimulation of E-selectin expression on endothelial cells with gp39-mCD8. Human umbilical vein endothelial cells (HUVEC Clonetics Corp., San Diego, CA) were treated with G28-5 sFv, G28-5, gp39-mCD8, or combinations as indicated, for 4 hrs then tested for expression of E-selectin by ELISA. HUVEC were cultured and stimulated in M199 (Gibco) with additions to final concentrations as follows: 4 m*M* L-glutamine, 48.5 μg/ml penicillin, 80 μg/ml streptomycin, 1 m*M* sodium pyruvate (Sigma, St. Louis, MO), 90 μg/ml heparin (Sigma, St. Louis, MO), 30 μg/ml endothelial growth supplement (Collaborative Biomedical Products, Boston, MA), and 20% fetal bovine serum. Endothelial cells were cultured in flasks treated with 1% gelatin, and plated at 1.5×10^4 cells/well in flat-bottomed 96-well Costar tissue culture plates that had been coated with 1μg/ml fibronectin (Collaborative Biomedical Products, Boston, MA). Endothelial cells, used at passage 5, were stimulated 1 to 2 days after plating. E-selectin expression was measured as previously described.[16] Values are the mean of three points and error bars represent the standard deviation. Error bars are absent when the bar is smaller than the graph symbol.

fixed and analyzed by confocal microscopy (Figure 4). The sFv and the IgG caused extensive CD40 clustering (panels B and C), whereas the Fab fragments did not (panel D). Similar results were obtained using a B lymphoblastoid cell line, T51 (data not shown).

One of the intracellular events that occurs after CD40 crosslinking is the activation of NF-κB.[5] Figure 5 (left panel) shows that a 2 hr incubation with either gp39-mCD8, G28-5 IgG, G28-5 sFv, or LPS caused activation of NF-κB. However, in this assay, the G28-5 IgG and G28-5 sFv were required at 10-fold higher concentrations than gp39-mCD8 for equivalent activation. The levels of octamer-binding protein levels were not affected by any of the treatments, and gp39-mCD8 caused some nuclear localization of AP-1, whereas G28-5 IgG and sFv did not (data not shown). Figure 5 (right panel) shows that binding of G28-5 IgG activated NF-κB in RAJI cells, and this activation was effectively blocked with Fab fragments of G28-5. Therefore, the G28-5 sFv and G28-5 Fab differ in their ability to activate NF-κB in correlation to their agonistic or antagonistic activity.

II. DISCUSSION

Similar to other members of the TNFR family, it has been found that crosslinking of CD40 is a major determinant of signal transduction and functional responses mediated by the receptor.[18] In comparison to soluble gp39-mCD8, we previ-

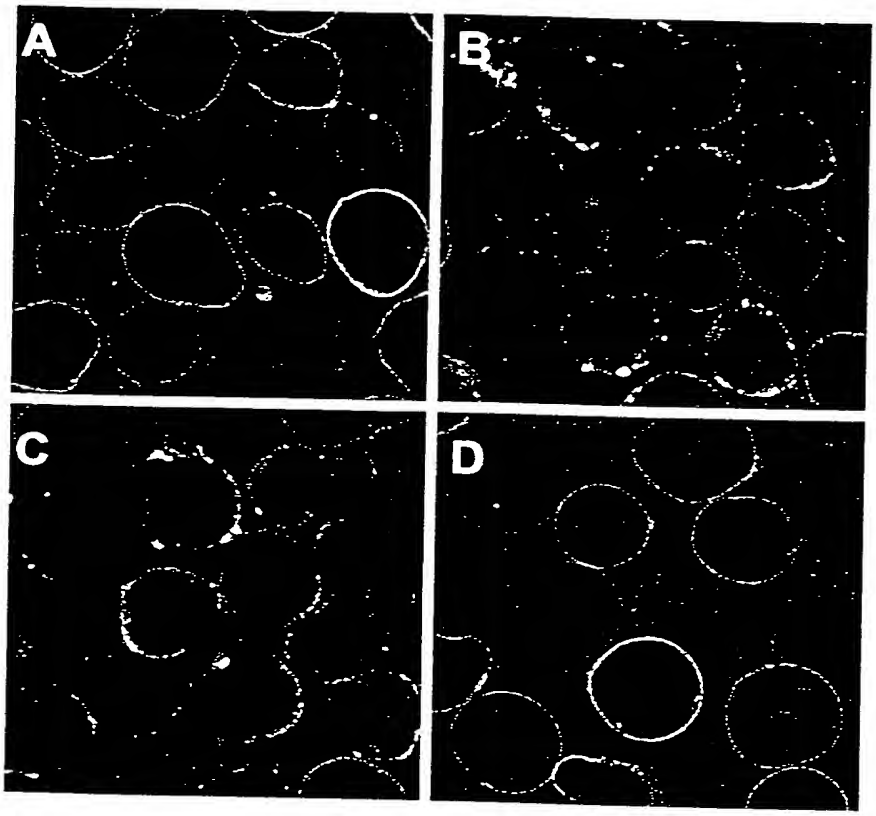


FIGURE 4. CD40 receptor aggregation on the cell surface induced by G28-5 sFv. (A) Raji B cells were incubated in media alone; (B) with G28-5 IgG; (C) with G28-5 sFv; (D) or with G28-5 Fab for 2 hrs at 37°C. Cells were then washed at 4°C and stained with fluorescein-conjugated CD40 mAb 1.87, followed by fixation with 1% paraformaldehyde and analysis using a Leica confocal microscope with a box oil objective.

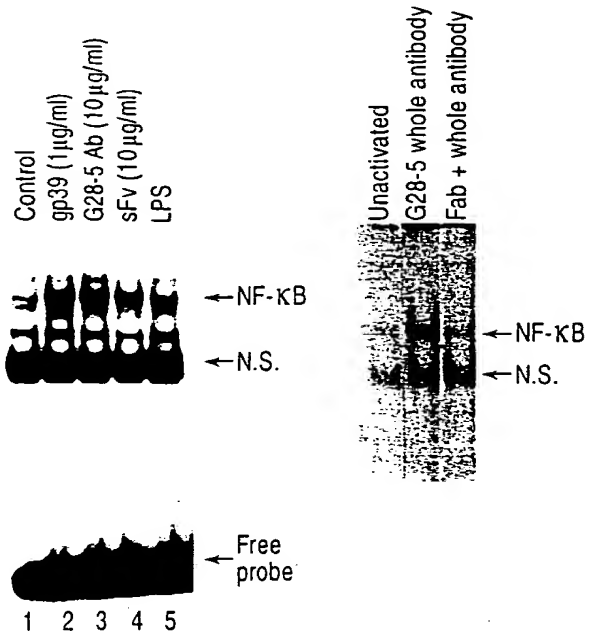


FIGURE 5. Activation of NF-κB in RAJI cells. Left panel: RAJI cells (1×10^7) were activated with either gp39-mCD8 (1 µg/ml), G28-5 mAb (10 µg/ml), G28-5 sFv (10 µg/ml), or LPS (1 µg/ml) for 2 hrs at 37°C. Right panel: RAJI cells were preincubated with 1 µg/ml G28-5 Fab prior to addition of G28-5 mAb (10 µg/ml). Nuclear extracts were prepared from approximately 5×10^6 cells using a modification of the procedure of Dignam et al.[25] Briefly, cells were lysed for 5 min. at 4°C in 10 m*M* Hepes, 1.5 m*M* $MgCl_2$, 10 m*M* NaCl, 0.25% NP-40, pH 7.5, for 30 min at 4°C. The nuclear extract was centrifuged at 14,000 rpm for 5 min, and the supernatants used for the electrophoretic gel mobility shift assay (EMSA), which was performed according to the procedure of Sen and Baltimore.[26] Approximately 3 µg of protein was incubated with a double-stranded ^{32}P-labeled kappa binding site containing the sequence: 5′ AGTTGAGGGGACTTTCCCAGG 3′ (Promega, Madison, WI), as described. Samples were analyzed on 6% polyacrylamide gels followed by autoradiography.

ously showed that G28-5IgG was both a partial agonist and a partial antagonist.[4] Extensive crosslinking of CD40 with mAbs plus second-step antiglobulins further enhanced B-cell proliferation responses.[19] Binding of CD40 mAbs to Fc receptors on transfected cells also enhanced CD40 functional responses and was most effective in prolonging B-cell proliferation and viability when used with IL4.[2] In addition, the CD40 ligand, like other TNF family members, is a trimer with the ligand binding sites formed from trimer junctions.[18,20] These studies show that the ability of the natural ligand to bind CD40 and to crosslink CD40 are inseparable. For these reasons we expected that a monovalent sFv binding to CD40 would not crosslink and would be a potential inhibitor of CD40 receptor signaling. In support of this idea a monovalent sFv to TNFRI that is an effective inhibitor of TNF-mediated cytotoxicity was recently described.[21]

Somewhat unexpectedly, we found that a monovalent G28-5 sFv was a more potent agonist than the parental bivalent mAb, even though the sFv was a 27-kDa monomer on SDS gels and a single peak of 16 kDa on nondenaturing gel filtration columns. The monomeric sFv bound to soluble CD40-Ig hexamers with a stochiometry close to 1 molecule sFv : 1 molecule CD40-Ig and unlike the whole mAb or the gp39-CD8 fusion protein, the sFv did not cause the formation of higher MW complexes in solution (data not shown). However, the sFv caused CD40 to rapidly aggregate on the cell surface, suggesting that the sFv activates CD40 responses through a crosslinking-dependent mechanism. Low concentrations of the G28-5 sFv directly stimulated homotypic adhesion of B cell or monocyte cell lines and synergized with either PMA, anti-CD20, or anti-IgM for stimulation of B-cell proliferation. Higher concentrations of the sFv (5 to 10 µg/ml) were needed to induce other responses in monocytes, including cytokine release, prevention of apoptosis, and stimulation of proliferation. In contrast, unlike gp39-mCD8, the sFv did not upregulate E-selectin expression on CD40-positive endothelial cells. Additionally, the sFv enhanced rather than inhibited gp39-mCD8 activity, and was able to overcome the inhibition by G28-5 IgG. These results show the monovalent sFv is a potent but partial agonist for the CD40 receptor and does not retain the antagonistic properties of G28-5 IgG. Interestingly, the activity of the sFv is unlike the Fab fragments of G28-5, because the Fab fragments retained very little agonistic activity (Table 1) and did not cause CD40 clustering on the cell surface (Figure 4).

To further understand the mechanism of signal transduction by G28-5 sFv, we examined the activation of NF-κB in the B-cell line RAJI. NF-κB translocation to the nucleus is known to occur

within 2 to 3 hours after CD40 binding either by G28-5 IgG or by natural ligand, and this response can be prevented by the tyrosine kinase inhibitor herbimycin A.[5] We found that crosslinking of CD40 was important for activation of NF-κB because a monovalent Fab fragment of G28-5 blocked the activity of the intact IgG. The sFv was inactive at low concentrations and could activate NF-κB only at high concentrations (10 μg/ml). Both gp39-mCD8 and G28-5 IgG were more potent than the sFv in stimulation of NF-κB translocation to the nucleus. These results suggest that the sFv is inefficient in activation of the signals required for translocation of NF-κB to the nucleus. Because the sFv can activate various cellular responses at low concentrations (10 ng/ml), yet NF-κB is only activated at much higher concentrations (10 μg/ml), it is likely that transcription factors other than NF-κB are activated by CD40 ligation. In fact, Francis et al.[22] have shown that AP-1 and NF-AT are activated by B-cell stimulation through the CD40 receptor.

The mechanism by which G28-5 sFv induces CD40 clustering on the cell surface is not known. It is possible that the sFv aggregates more readily in the membrane environment. We and others have observed that many sFv molecules have a strong tendency to aggregate.[23] However, it is also possible that other factors such as conformational changes in the sFv or in CD40 are involved. It is possible that partial agonists for CD40 derived from sFv molecules will be useful for application *in vivo*. Our data suggest that it is possible to make artificial receptor agonists with altered cell-type selectivity for CD40 activation, different from the natural ligand.

ACKNOWLEDGMENTS

We thank Teresa Nelson for help in preparation of the manuscript, Derek Hewgill for assistance with flow cytometry, and Sigrid Myrdal for help with confocal microscopy.

REFERENCES

1. **Clark, E. A. and Ledbetter, J. A.,** Activation of human B cells mediated through two distinct cell surface differentiation antigen, Bp35 and Bp50, *Proc. Natl. Acad. Sci. U.S.A.*, 83, 4494–4498, 1986.
2. **Banchereau, J., dePaoli, J., Valle, A., Garcia, E., and Rousset, F.,** Long-term human B cell lines dependent on interleukin-4 and antibody to CD40, *Science*, 251, 70, 1991.
3. **Banchereau, J., Bazan, F., Blanchard, D., Briere, F., Galizzi, J. P., van Kooten, C., Liu, Y. J., Rousset, F., and Saeland, S.,** The CD40 antigen and its ligand, *Annu. Rev. Immunol.*, 12, 881–922, 1994.
4. **Ledbetter, J. A., Grosmaire, L. S., Hollenbaugh, D., Aruffo, A., and Nadler, S. G.,** Agonistic and antagonistic properties of CD40 mAb G28-5 are dependent on binding valency, *Circ. Shock*, 44, 67–72, 1995.
5. **Berberich, I., Shu, G. L., and Clark, E. A.,** Crosslinking CD40 on B cells rapidly activates NF-κB, *J. Immunol.*, 153, 4357–4366, 1994.
6. **Engelmann, H., Holtmann, H., Brakebusch, C., Avni, Y. S., Sarov, I., Nophar, Y., Hadas, E., Leitner, O., and Wallach, D.,** Antibodies to a soluble form of a tumor necrosis factor (TNF) receptor have TNF-like activity, *J. Biol. Chem.*, 265, 14497–14504, 1990.
7. **Nagata, S. and Golstein, P.,** The Fas death factor, *Science*, 267, 1449–1456, 1995.
8. **Armitage, R. J.,** Tumor necrosis factor receptor superfamily members and their ligands, *Curr. Opin. Immunol.*, 6, 407–413, 1994.
9. **Noelle, R. J., Roy, M., Shepherd, D. M., Stamenkovic, I., Ledbetter, J. A., and Aruffo, A.,** A 39-KDa protein on activated helper T cells binds CD40 and transduces the signal for cognate activation of B cells, *Proc. Natl. Acad. Sci. U.S.A.*, 89, 6550–6554, 1992.
10. **Armitage, R. J., Strockbine, L., Sato, T. A., Clifford, K. N., Macduff, B. M., Anderson, D. M., Gimpel, S. D., Davis-Smith, T., Maliszewski, C. R., Clark, E. A., et al.,** Molecular and biological characterization of a murine ligand for CD40, *Nature*, 357, 80–82, 1992.
11. **Hollenbaugh, D., Grosmaire, L. S., Kullas, C. D., Chalupny, N. J., Braesch-Andersen, S., Noelle, R. J., Stamenkovic, I., Ledbetter, J. A., and Aruffo, A.,** The human T cell antigen gp39, a member of the TNF gene family, is a ligand for the CD40 receptor: expression of a soluble form of gp39 with B cell co-stimulatory acitivity, *EMBO J.*, 11, 4313–4321, 1992.
12. **Francisco, J. A., Gilliland, L. K., Stebbins, M. R., Norris, N. A., Ledbetter, J. A., and Siegall, C. B.,** Activity of a single-chain immunotoxin that selectively kills lymphoma and other B-lineage cells expressing the CD40 antigen, *Cancer Res.*, 55, 3099–3104, 1995.
13. **Friedman, P. N., McAndrew, S. J., Gawlak, S. L., Chace, D., Trail, P. A., Brown, J. P., and Siegall, C. B.,** BR96 sFv-PE40, a potent single-chain immunotoxin that selectively kills carcinoma cells, *Cancer Res.*, 53, 334–339, 1993.
14. **Kiener, P. A., Moran-Davis, P., Rankin, B. M., Wahl, A. F., Aruffo, A., and Hollenbaugh, D.,** Stimulation of CD40 with purified soluble gp39 induces

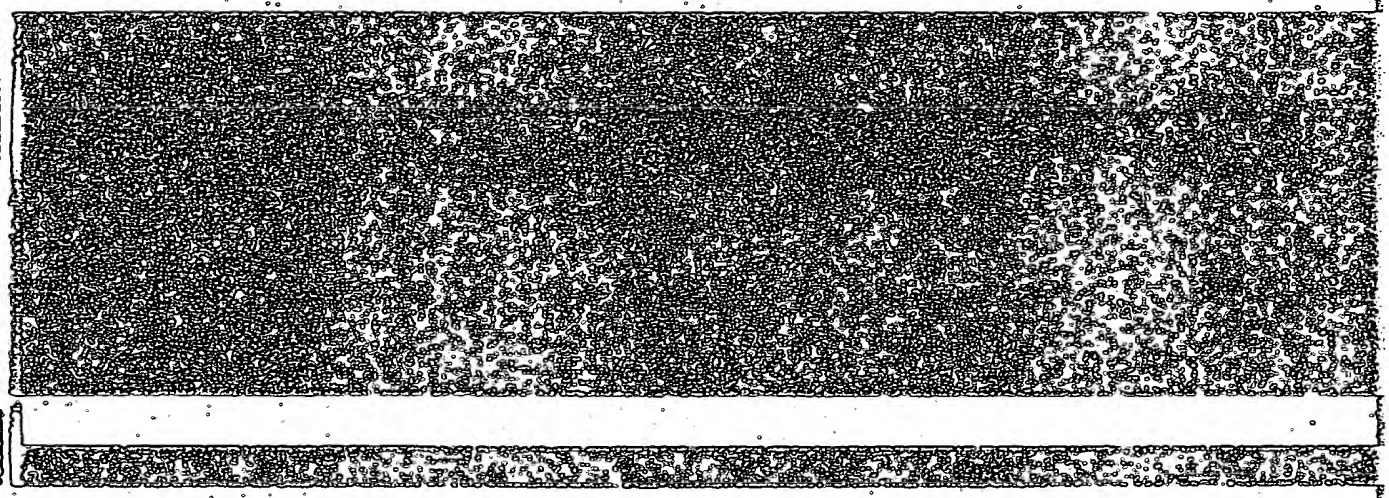
proinflammatory responses in human moncytes, *J. Immunol.*, 155, 4917–4925, 1995.

15. **Alderson, M. R., Armitage, R. J., Tough, T. W., Strockbine, L., Fanslow, W. C., and Spriggs, M. K.,** CD40 expression by human monocytes: regulation by cytokines and activation of monocytes by the ligand CD40, *J. Exp. Med.*, 178, 669–674, 1993.
16. **Hollenbaugh, D., Mischel-Petty, N., Edwards, C. P., Simon, J. C., Denfeld, R. D., Kiener, P. A., and Aruffo, A.,** Expression of functional CD40 by vascular endothelial cells, *J. Exp. Med.*, 182, 33–40, 1995.
17. **Karmann, K., Hughes, C. C. W., Schechner, J., Fanslow, W. C., and Pober, J. S.,** CD40 on human endothelial cells: inducibility by cytokines and functional regulation of adhesion molecule expression, *Proc. Natl. Acad. Sci. U.S.A.*, 92, 4342–4346, 1995.
18. **Van Ostade, X., Tavernier, J., and Fiers, W.,** Structure-activity studies of human tumour necrosis factors, *Pro Engl.*, 7, 5–22, 1994.
19. **Paulie, S., Rosen, A., Ehlin-Henriksson, B., Braesch-Andersen, S., Jakobson, E., Koho, H., and Perlmann, P.,** The human B lymphocyte and carcinoma antigen, CDw40, is a phosphoprotein involved in growth signal transduction, *J. Immunol.*, 142, 590–595, 1989.
20. **Bajorath, J., Chalupny, N. J., Marken, J. S., Siadak, A. W., Skonier, J., Gordon, M., Hollenbaugh, D., Noelle, R. J., Ochs, H. D., and Aruffo, A.,** Identification of residues on CD40 and its ligand which are critical for the receptor-ligand interaction, *Biochemistry*, 34, 1833–1840, 1995.
21. **Moosmayer, D., Dubel, S., Brocks, B., Watzka, H., Hampp, C., Scheurich, P., Little, M., and Pfizenmaier, K.,** A single-chain TNF receptor antagonist is an effective inhibitor of TNF mediated cytotoxicity, *Ther. Immunol.*, 2, 31–40, 1995.
22. **Francis, D. A., Karras, J. G., Ke, X., Sen, R., and Rothstein, T. L.,** Induction of the transcription factors NF-κB, AP-1 and NF-AT during B cell stimulation throughout the CD40 receptor, *Int. Immunol.*, 7(2), 151–161, 1995.
23. **Gilliland, L. K., Norris, N. A., Marquardt, H., Tsu, T. T., Hayden, M. S., Neubauer, M. G., Yelton, D. E., Mittler, R. S., and Ledbetter, J. A.,** Rapid and reliable cloning of antibody variable regions and generation of recombinant single chain antibody fragments, *Tissue Antigens*, 47, 1–20, 1996.
24. **Ledbetter, J. A. and Clark, E. A.,** Surface phenotype and function of tonsillar germinal center and mantle zone B cell subsets, *Hum. Immunol.*, 15, 30, 1986.
25. **Dignam, J. D., Lebovitz, R. M., and Roeder, R. G.,** Accurate transcription initiation by RNA polymerase II in a soluble extract from isolated mammalian nuclei, *Nucleic Acids Res.*, 11, 1475, 1983.
26. **Sen, R. and Baltimore, D.,** Multiple nuclear factors interact with the immunoglobulin enhancer sequences, *Cell*, 46, 705, 1986.

Pharmaceutical Biotechnology • Volume 6

Vaccine Design

The Subunit and Adjuvant Approach



Edited by
Michael F. Powell
and
Mark J. Newman

Chapter 7

A Compendium of Vaccine Adjuvants and Excipients

Frederick R. Vogel and Michael F. Powell

In the early 20th century, researchers experimented with diverse compounds such as alum (aluminum salts), mineral oil, and killed mycobacteria to improve the immunogenicity of vaccines. These first empirical studies demonstrated the adjuvant activity of many substances, but several products also elicited significant local and systemic adverse reactions that precluded their use in human vaccine formulations. Alum adjuvant, first described in 1926, remains the only immunologic adjuvant used in human vaccines licensed in the United States.

Since the advent of modern immunology twenty years ago, hundreds of immunomodulatory compounds have been evaluated as vaccine adjuvants. After extensive safety and toxicity testing, many of these modern adjuvants have proven to be acceptable for clinical evaluation. During the same time, investigations into the mechanisms of action of adjuvants have increased. Today, a major goal of adjuvant research is to apply the increased understanding of adjuvant activities so adjuvants can be selected based on the immune response desired for a particular vaccine.

The purpose of this compendium is to provide a reference for investigators interested in accessing information on the variety of adjuvants available for study, and to foster collaboration between basic and applied vaccine researchers, and adjuvant developers. This compendium is extensive but by no means complete. It is our hope that vaccinologists will find this a useful resource and that it may help to advance adjuvant development as an integral part of a rational vaccine design.

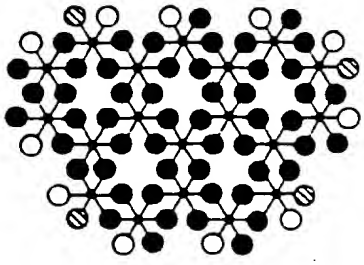
Frederick R. Vogel • Division of AIDS, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland 20892. *Michael F. Powell* • Pharmaceutical Research & Development, Genentech, Inc., South San Francisco, California 94080.

Vaccine Design: The Subunit and Adjuvant Approach, edited by Michael F. Powell and Mark J. Newman, Plenum Press, New York, 1995.

COMPONENT/ADJUVANT NAME: Adju-Phos

OTHER NAME(S): Aluminum phosphate gel

STRUCTURE: Amorphous aluminum hydroxyphosphate. A schematic of the unit layer of amorphous aluminum hydroxyphosphate showing the surface hydroxyl, water, and phosphate groups. Key: Al, •; OH, ●; H_2O, ○; PO_4, ⊘



SOURCE: Obtained by precipitation. The degree of substitution of phosphate for hydroxyl depends on the concentration of reactants and precipitation conditions.

USES: Human applications: diphtheria, tetanus, and pertussis vaccines. Veterinary vaccine applications.

APPEARANCE: White gelatinous precipitate in aqueous suspension.

MOLECULAR WEIGHT: Not applicable.

RECOMMENDED STORAGE: 4–25°C. Never expose to freezing. Recommended 2 year shelf life.

CHEMICAL/PHYSICAL PROPERTIES: Primary particles have a platelike morphology and a diameter of 50–100 nm. The isoelectric point is acidic and is inversely related to the degree of substitution of phosphate for hydroxyl. Its high surface area gives it a high adsorptive capacity for antigens. Particle size range of final product 0.5–10 μm.

INCOMPATIBILITY: Dissolves in strong bases and acids.

SAFETY/TOXICITY: May cause mild local reactions at the site of injection (erythema and/or mild transient swellings).

- Yamanaka, M. *et al.*, 1992, Pathological studies on local tissue reactions in guinea pigs and rats caused by four different adjuvants, *J. Vet. Med. Sci.* **54**:685–692.
- Gupta, R. K., *et al.*, 1993, Adjuvants—A balance between toxicity and adjuvanticity, *Vaccine* **11**:293–306.

ADJUVANT PROPERTIES: The surface area, surface charge, and morphology of the amorphous aluminum hydroxyphosphate are major factors in its adjuvant characteristics. The use of aluminum adjuvants is accompanied by stimulation of IL-4 and stimulation of the T-helper-2 subsets in mice, with enhanced IgG1 and IgE production. Properties are described in:

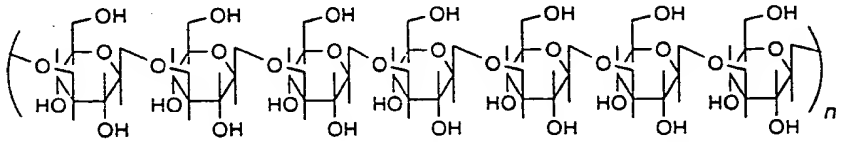
- Seeber, S., *et al.*, 1991, Predicting the adsorption of proteins by aluminum-containing adjuvants, *Vaccine* **9**:201–203.
- Shirodkar, *et al.*, 1990, Aluminum compounds used as adjuvants in vaccines, *Pharm. Res.* **7**:1282–1288.
- Seeber, S. J., *et al.*, 1991, Solubilization of aluminum-containing adjuvants by constituents of interstitial fluid, *J. Parenteral Sci. Tech.* **45**:156–159.
- Gupta, R. K., *et al.*, Chapter 8, this volume.

CONTACT(S): E. B. Lindblad, Superfos Biosector a/s, DK-2950 Vedbaek, Denmark, Ph: 45 42 89 31 11; Fax: 45 42 89 15 95. Also: Al Reisch, Sergeant, Inc., Clifton, NJ 07012, Ph: 201-472-9111; Fax: 201-472-5686. Also: Stanley Hem, Purdue University, W. Lafayette, IN 47907-1336, Ph: 317-494-1451; Fax: 317-494-7880.

COMPONENT/ADJUVANT NAME: Algal Glucan

OTHER NAME(S): β-glucan; glucan

STRUCTURE: A linear β-D(1,3)-linked glucopyranose polymer having a triple-helical conformation.



SOURCE: Produced by an adapted strain of *Euglena gracilis* (SRI strain D86-G) grown heterotrophically in the dark. Obtained from the cytoplasm of the organism by methanol and chloroform extraction. Depyrogenized in hot 1 N HCl and washed sequentially in pyrogen-free water and pyrogen-free saline.

- Tusè, D., *et al.*, 1992, Production of β-1,3-glucan in *Euglena*, U.S. Patent No. 5,084,386.

USES: Administered with antigen for enhancement of both humoral and cell-mediated immunity. β-Glucans exert their immunostimulatory activities by binding to specific β-glucan receptors on macrophages. This ligand–receptor interaction results in macrophage activation and, in certain formulations, promotes antigen targeting.

- DiLuzio, N. R., *et al.*, 1979, Evaluation of the mechanism of glucan-induced stimulation of the reticuloendothelial system, *J. Reticuloendothel. Soc.* 7:731–742.
- Czop, J. K., and Austen, K. F., 1985, A β-glucan inhibitable receptor on human monocytes: Its identity with the phagocytic receptor for particulate activators of the alternative complement pathway, *J. Immunol.* 134:2588–2593.

APPEARANCE: White, odorless crystalline material. Forms a suspension in aqueous solutions.

MOLECULAR WEIGHT: Highest measured MW = 500,000.

RECOMMENDED STORAGE: Stable to light. Store solid Algal Glucan at room temperature and aqueous suspensions at 4°C. No apparent degradation after storage of aqueous suspension for 24 months at 4°C. Optimal storage conditions are to be determined.

CHEMICAL/PHYSICAL PROPERTIES: Native particulate material is water insoluble. Median particle size 3.7–4.6 μm, with specific gravity of 1.86–2.0 g/cm^3. Purified preparations contain 0.0001–0.35% phosphorus and 0.12–0.27% nitrogen.

INCOMPATIBILITY: Alkaline pH disrupts the triple-helical conformation.

SAFETY/TOXICITY: In preclinical studies, Algal Glucan has been intravenously administered at doses up to 25 mg/kg body weight and was well tolerated. Human clinical trials of β-glucans isolated from either plants or microorganisms indicate the feasibility of administering these compounds to humans without toxicity. Glucan particles bioerode over time in a physiological environment.

- Mansel, P. W. A., *et al.*, 1975, Macrophage-mediated destruction of human malignant cells in vivo, *J. Natl. Cancer Inst.* 54:571–580.
- Okamura, K., *et al.*, 1986, Clinical evaluation of *Schizophyllan* combined with irradiation in patients with cervical cancer, *Cancer* 58:865–872.
- Chihara, G., *et al.*, 1989, Lentinan as a host defense potentiator (HDP), *Int. J. Immunother.* 4:145–154.
- Ostroff, G. R., 1994, Future therapeutic applications of Betafectin, a carbohydrate-based immunomodulator, The Second Annual Conference on Glycotechnology.

ADJUVANT PROPERTIES: Algal Glucan, a nonantigenic carbohydrate adjuvant, enhances both humoral and cell-mediated immunity to oligopeptides in experimental animal

models. Mice immunized twice by coadministration of herpes virus glycoprotein D (gD2) and 100 μg Algal Glucan produced anti-gD2 antibodies that were significantly higher in titer and persisted longer ($p<0.01$) than those in animals injected with gD2 alone. Similarly, immunization of mice with either gD2- or HIV-1-gp120 with Algal Glucan added as an adjuvant heightened the antigen-specific response of splenic lymphocytes.

• Mohagheghpour, N., *et al.*, 1992, Adjuvant activity of an algal glycan, VIII International Conference on AIDS/III STD World Congress, Amsterdam.

CONTACT(S): Dr. Nahid Mohagheghpour, SRI International, Menlo Park, CA 94025. Ph: 415-859-3516; Fax: 415-859-3342. Also: Richard McIntosh, Genesis Technology Group, Inc., Cambridge, MA, Ph: 617-576-6610; Fax: 617-876-4002.

COMPONENT/ADJUVANT NAME: Algammulin

OTHER NAME(S): Gamma inulin/alum composite adjuvant

STRUCTURE: See entries under gamma inulin and Alhydrogel for primary materials. Inulin is crystallized in presence of Alhydrogel suspensions and transformed to gamma inulin at 37°C to form electron-dense ovoids that both adsorb antigen and activate complement.

- Cooper, P. D., and Steele, E. J., 1991, Algammulin: A new vaccine adjuvant comprising gamma inulin particles containing alum, *Vaccine* **9**:351–357.

USES: Included in adjuvant formulations as a primary adjuvant.

APPEARANCE: Milky white, nonviscous aqueous suspension, easily resuspended. Supplied at 50 mg/mL, sterile and pyrogen-free.

MOLECULAR WEIGHT: See entries under gamma inulin and Alhydrogel.

RECOMMENDED STORAGE: 2–8°C; maintain in aqueous medium. Do not freeze or heat over 45°C.

CHEMICAL/PHYSICAL PROPERTIES: See entries under gamma inulin and Alhydrogel for primary materials. Algammulin is stable for years under recommended storage. Unstable below pH 6 and above pH 10. Virtually insoluble at 37°C.

INCOMPATIBILITY: Degraded in strong acid. Adjuvants containing aluminum hydroxide gel may be incompatible with phosphate or anionic detergents.

SAFETY/TOXICITY: Nonpyrogenic, nonantigenic, and of very low toxicity in experimental animals and a Phase I clinical trial. Biodegradable to simple sugars and aluminum hydroxide gel. Large intravenous doses can cause acute complement-activation shock similar to that sometimes found in renal dialysis patients. Dissolved inulin is pharmacologically inert and is registered for human use; alum is also approved for human use.

ADJUVANT PROPERTIES: Expected to stimulate immune responses by causing ligation of leukocyte-surface complement receptors (CR) via known biochemical mechanisms, thus placing the antigen close to activated leukocytes. Addition of Algammulin is known to enhance both humoral and cell-mediated immunity from either Th1 or Th2 pathways, depending on the weight ratio of inulin to Alhydrogel.

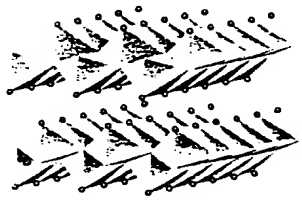
- Cooper, P. D., *et al.*, 1991, The adjuvanticity of Algammulin, a new vaccine adjuvant, *Vaccine* **9**:408–415.
- Cooper, P. D., *et al.*, 1993, Gamma inulin and Algammulin: Two new vaccine adjuvants, in: *Vaccines 93, Modern Approaches to New Vaccines Including Prevention of AIDS* (H. S. Ginsburg, F. Brown, R. M. Chanock, and R. A. Lerner, eds.), Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, pp. 25–30.
- Cooper, P. D., Chapter 24, this volume.

CONTACT(S): Dr. Peter D. Cooper, Division of Cell Biology, John Curtin School of Medical Research, Australian National University, Canberra, A. C. T., Australia 2601, Ph: 61-6-291-8670; Fax: 61-6-249-2595.

COMPONENT/ADJUVANT NAME: Alhydrogel

OTHER NAME(S): Aluminum hydroxide gel; alum

STRUCTURE: Crystalline aluminum oxyhydroxide AlOOH, known mineralogically as boehmite. The structure consists of corrugated sheets of aluminum octahedra.



SOURCE: Obtained by precipitation of aluminum hydroxide under alkaline conditions.

USES: Human applications: diphtheria, tetanus, and pertussis vaccines. Veterinary vaccine applications.

APPEARANCE: White gelatinous precipitate in aqueous suspension.

MOLECULAR WEIGHT: Not applicable.

RECOMMENDED STORAGE: 4–25°C. Never expose to freezing. Recommended 2 year shelf life.

CHEMICAL/PHYSICAL PROPERTIES: Primary particles have a rodlike or fibril morphology and a high surface area. The isoelectric point is 11. Its high surface area gives it a high adsorptive capacity for antigen. Poorly soluble in solutions containing citrate ions. Normal particle size range 0.5–10 μm.

INCOMPATIBILITY: Dissolves in strong bases and acids.

SAFETY/TOXICITY: May cause mild local reactions at the site of injection (erythemas and/or mild transient swellings).

- Ganrot, P. O., 1986, Metabolism and possible health effects of aluminum, *Environ. Health Perspect.* **65**:363–441.
- Gupta, R. K., *et al.*, 1993, Adjuvants—A balance between toxicity and adjuvanticity, *Vaccine* **11**:293–306.

ADJUVANT PROPERTIES: Alhydrogel is the standard preparations for immunological research on aluminum hydroxide gels. The use of aluminum adjuvants is accompanied by stimulation of IL-4 and stimulation of the T-helper-2 subsets in mice, with enhanced IgG1 and IgE production. Further immunomodulation is accomplished by the aluminum content. Properties are described in:

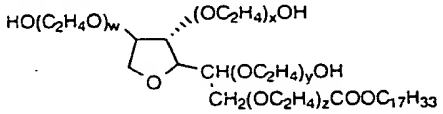
- Shirodkar, S., *et al.*, 1990, Aluminum compounds used as adjuvant in vaccines, *Pharm. Res.* **7**:1282–1288.
- Stewart-Tull, D. E. S., 1989, Recommendations for the assessment of adjuvants (immunomodulators), in: *Immunological Adjuvants and Vaccines* (Gregoriadis, G., Allison, A. C., and Poste, G., eds.), Plenum Press, New York, pp. 213–226.
- Gupta, R., *et al.*, Chapter 8, this volume.
- Seeber, S., *et al.*, 1991, Predicting the adsorption of proteins by aluminum-containing adjuvants, *Vaccine* **9**:201–203.
- Seeber, S. J., *et al.*, 1991, Solubilization of aluminum-containing adjuvants by constituents of interstitial fluid, *J. Parenteral Sci. Tech.* **45**:156–159.
- Hem, S., and White, J. L., Chapter 9, this volume.

CONTACT(S): E. B. Lindblad, Superfos Biosector a/s, DK-2950 Vedbaek, Denmark, Ph: 45 42 89 31 11; Fax: 45 42 89 15 95. Also: Al Reisch, Sergeant, Inc., Clifton, NJ 07012, Ph: 201-472-9111; Fax: 201-472-5686. Also: Stanley Hem, Purdue University, West Lafayette, IN 47907-1336, Ph: 317-494-1451; Fax: 317-494-7880.

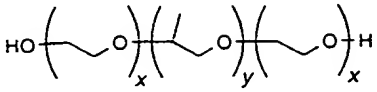
COMPONENT/ADJUVANT NAME: Antigen Formulation

OTHER NAME(S): SPT, AF

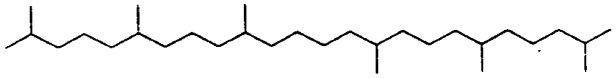
STRUCTURE: An emulsion of squalane (5%), Tween 80 (0.2%), Pluronic L121 (1.25%), phosphate-buffered saline pH 7.4, and antigen.



Polysorbate 80



Pluronic L121



Squalane

SOURCE: Oil-in-water microemulsion obtained by the microfluidization of the components at reduced temperature.

USES: A vaccine adjuvant vehicle that, when administered with antigen, induces both a cellular and humoral immune response.

APPEARANCE: Homogeneous, white milky liquid.

MOLECULAR WEIGHT: Not applicable.

RECOMMENDED STORAGE: 2–8°C under inert gas. Avoid freezing.

CHEMICAL/PHYSICAL PROPERTIES: A microemulsion comprised of oil droplets of mean diameter around 150–175 nm. Vialed as a 3X formulation, AF is stable for up to 2 years when stored at 5°C, depending on the concentrations of the excipients used as well as the conditions of microfluidization. A uniform dispersion is achieved when diluting 1:3 with aqueous solution prior to administration.

INCOMPATIBILITY: None found.

SAFETY/TOXICITY: Pathology and toxicology studies completed in two species, including nonhuman primates. It is well tolerated at doses and schedules that exhibit immune stimulating activity. The safety and potency of the three-component microfluidized formulation has been demonstrated in Phase I/II clinical trials.

ADJUVANT PROPERTIES: Gives good humoral and CTL responses. A potent cytotoxic T cell response was induced when recombinant soluble antigens were injected with AF leading to the destruction of tumor cells or virally infected cells in vitro and in vivo.

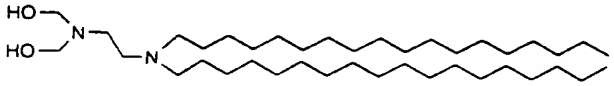
- Raychaudhuri, *et al.*, 1992. Induction of antigen-specific class I-restricted cytotoxic T cells by soluble proteins in vivo. *Proc. Natl. Acad. Sci. USA* **89**:8308–8312.

CONTACT(S): Thomas Ryskamp, IDEC Pharmaceuticals Corporation, San Diego, CA 92121, Ph: 619-550-8500; Fax: 619-550-8750; Internet: tryskamp@idec.com.

COMPONENT/ADJUVANT NAME: Avridine®

OTHER NAME(S): *N,N*-dioctadecyl-*N'*,*N'*-bis(2-hydroxyethyl) propanediamine; CP-20,961

STRUCTURE:



SOURCE: Chemical synthesis.

USES: Incorporation into a liposomal preparation, e.g., at a molar ratio of 1:2 Avridine:dimyristoyl phosphatidylcholine forms unilamellar liposomes; aqueous suspensions from alcoholic solution; in Intralipid, an aqueous soybean oil emulsion vehicle; other vegetable and mineral oil vehicles; Tween 80 dispersions in saline; saline suspension with alum-precipitated antigen.

APPEARANCE: White powder.

MOLECULAR WEIGHT: 667.17

RECOMMENDED STORAGE: Store as a powder at room temperature.

CHEMICAL/PHYSICAL PROPERTIES: Very insoluble in water, exhibits waxy properties at temperatures below 39°C; good solubility in absolute ethanol.

INCOMPATIBILITY: None known.

SAFETY/TOXICITY: Intranasal administration to humans induces interferon in nasal secretions and protection against rhinovirus challenge; injection site irritations; model for adjuvant arthritis in Lewis rats; antitumor properties in rodent tumor models.

- Niblack, J. F., 1977, Studies with low molecular weight inducers of interferon in man, *Toxicol. Rep. Biol. Med.* 35:528–534.
- Waldman, R. H., and Ganguly, R., 1978, Effect of CP-20,961, an interferon inducer, on upper respiratory tract infections due to rhinovirus type 21 in volunteers, *J. Infect. Dis.* **138**:531–535.
- Chang, Y. H., *et al.*, 1980, Adjuvant polyarthritis. IV. Induction by a synthetic adjuvant: Immunologic, histopathologic, and other studies, *Arthritis Rheum.* **23**:62–71.

ADJUVANT PROPERTIES: Humoral and cellular immunity, proliferation of B and T lymphocytes, protective immunity, activation of macrophages, induction of interferon, enhancement of mucosal immunity when administered orally/enterically with antigen, adjuvanticity with a variety of antigens, induction of IgG2a and IgG2b isotypes.

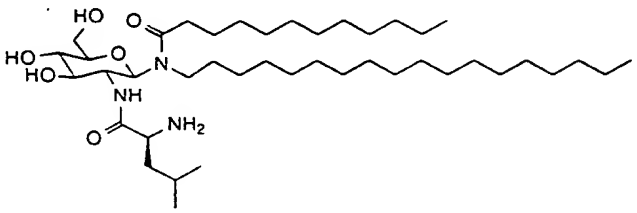
- Niblack, J. F., *et al.*, 1979, CP-20,961: A structurally novel, synthetic adjuvant, *J. Reticuloendothel. Soc.* **26**(Suppl.):655–666.
- Kraaijeveld, C. A., *et al.*, 1982, Enhancement of delayed-type hypersensitivity and induction of interferon by the lipophilic agents DDA and CP-20,961, *Cell. Immunol.* **74**:277–283.
- Jensen, K. E., 1988, Synthetic adjuvants: Avridine and other interferon inducers, in: *Advances in Carriers and Adjuvants for Veterinary Biologics* (R. M. Nerwig, P. M. Gough, M. L. Kaeber, and C. A. Whetstone, eds.), Iowa State University Press, Ames, pp. 79–89.
- Anderson, A. O., *et al.*, 1987, Studies on anti-viral mucosal immunity with the lipoidal amine adjuvant Avridine, *Adv. Exp. Med. Biol.* **216B**:1781–1790.

CONTACT(S): Dr. Oksana K. Yarosh, VIDO, Saskatoon, Canada S7N 0W0, Ph: 306-966-7465; Fax: 306-966-7478; E mail: yarosh@sask.usask.ca. Also: Huw Hughes, M6 Pharmaceuticals, Inc., New York, NY 10701, Ph: 212-308-7200 ext. 14; E-mail: 74577.345@ compuserve.com.

COMPONENT/ADJUVANT NAME: BAY R1005

OTHER NAME(S): *N*-(2-Deoxy-2-L-leucylamino-β-D-glucopyranosyl)-*N*-octadecyl-dodecanoylamide hydroacetate

STRUCTURE:



SOURCE: Chemical synthesis. Provided as the acetate salt.

- Lockhoff, O., 1991, Glycolipids as immunomodulators: Synthesis and properties, *Angew. Chem. Int. Ed. Engl.* **30**:1611–1620.

USES: Primary adjuvant.

APPEARANCE: White lyophilizate.

MOLECULAR WEIGHT: 726.1 + Acetate 60.1

RECOMMENDED STORAGE: Store at 2–8°C in airtight containers.

CHEMICAL/PHYSICAL PROPERTIES: Slightly hygroscopic. No polymorphism detected. Chemically stable to air, light, at temperatures up to 50°C, and in aqueous solvents at pH 2–12 at ambient temperature. Amphiphilic molecule, forms micelles in aqueous solution. Formation of translucent liposomal dispersion by ultrasonic treatment.

INCOMPATIBILITY: None found.

SAFETY/TOXICITY: Exploratory studies in rats (doses 2.5, 25, or 100 mg/kg body weight) on acute i.p. toxicity according to "OECD Guidelines for Testing Chemicals, No. 401": no deaths, $LD_{50} > 100$ mg/kg b.w. subacute i.v. toxicity in rats (doses 2.5, 25, or 100 mg/kg body weight) for 14 days with subsequent 4-week follow-up period to test reversibility of possible effects: no-effect level at 2.5 mg/kg b.w.

ADJUVANT PROPERTIES: BAY R1005 in combination with purified virus vaccines or subunit vaccines led to increased protection of virus-challenged mice. Preclinical trials in other animal species (pig, sheep, horse) gave comparable results with respect to antibody production. The increase in antibody synthesis induced by BAY R1005 is specifically dependent on the antigen and is not the result of polyclonal stimulation. BAY R1005 acts on the proliferation of B lymphocytes as a second signal which has no effect until the antigen acts as a first signal. BAY R1005 is capable of activating B lymphocytes without the helper function of T lymphocytes.

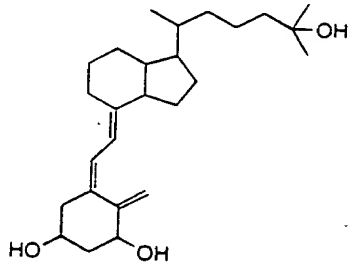
- Stünkel, K. G., *et al.*, 1988, In vitro studies of synthetic glycolipids: A new class of compounds with immunomodulating activity, in: *Leucocyte Activation and Differentiation* (J. C. Mani and J. Dorn, eds.), de Gruyter, Berlin, pp. 421–425.
- Stünkel, K. G., *et al.*, 1988, Synthetic glycolipids: In vitro characterization of a new class of compounds with immunomodulating properties, *Adv. Biosci. (Oxford)* **68**:429–437.
- Stünkel, K. G., *et al.*, 1989, Synthetic glycolipids with immunopotentiating activity on humoral immunity: Evaluation in vivo, *Prog. Leukocyte Biol.* **9**:575–579.

CONTACT(S): Dr. O. Lockhoff, Bayer AG, D-51368 Leverkusen, Germany, Ph: 49-214-30-7958; Fax: 49-214-30-50070.

COMPONENT/ADJUVANT NAME: Calcitriol

OTHER NAME(S): 1α,25-dihydroxyvitamin D_3; 1,25-di$(OH)_2D_3$; 1,25-DHCC; 1α,25-dihydroxycholecalciferol; 9,10-seco(5Z,7E)-5,7,10(19)-cholestatriene-1α,3β,25-triol

STRUCTURE:



SOURCE: Roche (Nutley, NJ) is the principal supplier to academic researchers. For citations involving the initial identification and methods of preparation see the *Merck Index* (Merck and Co., Inc., Rahway, NJ) under the entry for calcitriol.

USES: Promotes the induction of mucosal immunity when incorporated into vaccine formulations.

APPEARANCE: White, colorless powder or crystalline material.

MOLECULAR WEIGHT: 416.65

RECOMMENDED STORAGE: Air and light sensitive. Storage in a dry inert atmosphere at or below –20°C.

CHEMICAL/PHYSICAL PROPERTIES: Calcitriol is a hydrophobic molecule with limited solubility in water. It is soluble in organic solvents including alcohols. Solutions should be prepared in glass to avoid losses of the compound to plastic surfaces. Melting point: 111–115°C. Ultraviolet absorption maximum in ethanol is 264 nm (e = 19,000).

INCOMPATIBILITY: Avoid combining calcitriol with components capable of addition or oxidation reactions involving the conjugated *p* electron system. In particular, components capable of releasing free halogens should be avoided.

SAFETY/TOXICITY: Because calcitriol is the active form of vitamin D it should not be given to patients with hypercalcemia. Safety, toxicity, known metabolites, and dosage data are summarized under the entry for Rocaltrol in the *Physicians Desk Reference* (Medical Economics Data Production Co., Montvale, NJ).

ADJUVANT PROPERTIES: The incorporation of calcitriol (0.1–1.0 μg) directly into vaccine formulations containing protein or polysaccharide antigens promotes the induction of both systemic and common mucosal immune responses. Hormone modulation with immunization eliminates the need to apply immunizing antigens to mucosal surfaces for the induction of secretory antibodies.

- Daynes, R. A., *et al.*, 1994. Cytokine modulation in vivo with vitamin D3: Promotion of common mucosal immunity following a standard subcutaneous vaccination, *FASEB J.* **8**:A283.
- Daynes, R. A., and Araneo, B. A., 1994. The development of effective vaccine adjuvants employing natural regulators of T-cell lymphokine production in vivo, *Ann. N.Y. Acad. Sci.* **730**:144–161.
- Daynes, R. A., *et al.*, 1995. Steroids as regulators of the mammalian immune response, *J. Invest. Derm.* (in press).

CONTACT(S): Raymond A. Daynes, Department of Pathology, University of Utah Medical Center, Salt Lake City, UT 84132, Ph: 801-581-3013; Fax: 801-581-8946.

COMPONENT/ADJUVANT NAME: Calcium Phosphate Gel

OTHER NAME(S): Calcium phosphate

STRUCTURE: Hydrated calcium phosphate gel.

SOURCE: Precipitated by mixing soluble calcium and phosphate salts under carefully controlled conditions.

USES: Calcium phosphate has been used as adjuvant in vaccine formulations against diphtheria, tetanus, pertussis, and poliomyelitis. It has also been used for adsorption of allergenic extracts for hyposensitization of allergic patients.

APPEARANCE: White, gelatinous precipitate in aqueous suspension.

MOLECULAR WEIGHT: Not applicable.

RECOMMENDED STORAGE: 4–25°C. Never expose to freezing.

CHEMICAL/PHYSICAL PROPERTIES: Adsorbs soluble antigens and presents them in a particulate form to the immune system. Normal particle size range 0.5–15 μm.

INCOMPATIBILITY: Maintain neutral pH.

SAFETY/TOXICITY: Calcium phosphate adjuvant contains no components that are not natural constituents of the body and is very well tolerated.

- Gupta, R. K., *et al.*, 1993, Adjuvants—A balance between toxicity and adjuvanticity, *Vaccine* **11**:293–306.

ADJUVANT PROPERTIES: Properties are described in:

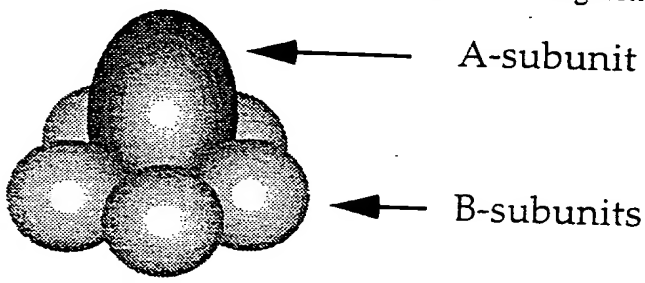
- Relyveld, E. H., 1986, Preparation and use of calcium phosphate-adsorbed vaccines, *Dev. Biol. Stand.* **65**:131–136.
- Relyveld, E. H., *et al.*, 1985, Calcium phosphate adjuvanted allergens, *Ann. Allergy* **54**:521–529.
- Gupta, R., *et al.*, Chapter 8, this volume.

CONTACT(S): E. B. Lindblad, Superfos Biosector a/s, DK-2950 Vedbaek, Denmark, Ph: 45 42 89 31 11; Fax: 45 42 89 15 95. Also: Al Reisch, Sergeant, Inc., Clifton, NJ 07012, Ph: 201-472-9111; Fax: 201-472-5686.

COMPONENT/ADJUVANT NAME: Cholera Holotoxin (CT)
Cholera Toxin B Subunit (CTB)

OTHER NAME(S): CT; CT B subunit; CT-B

STRUCTURE: The CT protein is composed of one enzymatically active, toxic A1 subunit which is linked to a pentamer of CTB subunits via the CTA2 fragment.



SOURCE: Bacterial protein is produced by *Vibrio cholerae*. CTB is the toxoid lacking the A subunit. Recombinant CTB is available. Suppliers include: List Biological Labs, Campbell, CA; Sigma Chemical Co, St. Louis, MO; Swedish National Vaccine Company, Stockholm, Sweden.

USES: CT is the prototype for an ADP-ribosylating bacterial toxin. It binds with high affinity via the CTB to its receptor ganglioside GM1 present on most mammalian cells. It is enzymatically active through its A1 subunit. The immunomodulating effect of CT is associated with the ADP-ribosylating ability, whereas effective delivery of antigen is achieved with the CTB as the carrier molecule. CT is the most effective adjuvant for mucosal immunity yet described for experimental use. CT in microgram doses may also be used experimentally to adjuvant systemic immune responses. Both humoral and cell-mediated immunity are known to be greatly augmented by CT-adjuvant. CT and CTB are used in soluble form simply by admixing with unrelated protein antigen or more effective as chemical conjugates with unrelated protein antigen. The CTB may be used as an efficient carrier molecule for other proteins or peptide fragments either chemically or genetically linked to CTB. CTB may be used in humans as an adjuvant/carrier molecule. Both CT and CTB are potent stimulators of immunological memory.

APPEARANCE: White lyophilized powder.

MOLECULAR WEIGHT: CT is 86,000, consisting of the A1 subunit (23,000), the CTA2 (5,000), and five subunits of CTB (11,000 each).

RECOMMENDED STORAGE: Store CT as a lyophilized powder under low humidity at 4°C. After reconstitution with water, store CT at –70°C, and CTB at 4°C.

CHEMICAL/PHYSICAL PROPERTIES: Good water solubility at neutral pH.

INCOMPATIBILITY: None found. Avoid proteases.

SAFETY/TOXICITY: CTB is nontoxic and has been used in humans without negative side effects. CT has not been used in humans because of its toxic effects, even at doses lower than 5 μg.

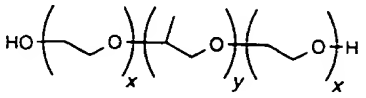
ADJUVANT PROPERTIES: CT exerts immunomodulating effects on T cells, B cells as well as antigen-presenting cells. Which of these effects is critical for adjuvant function is presently unknown. CTB lacks immunoenhancing effects after oral or intravenous administration, but may augment humoral response after intranasal administration. Although CTB

is an effective carrier molecule, it appears that a small amount of ADP-ribosylation is required for an efficient adjuvant effect.

- Merrit, E., *et al.*, 1994, Crystal structure of cholera toxin B pentamer bound to receptor GM1-pentasaccharide, *Protein Sci.* **3**:166–175.
- Burnette, W. N., 1994, AB5 ADP-ribosylating toxins: Comparative anatomy and physiology, *Structure* **2**:151–158.
- Holmgren, J., *et al.*, 1994, Cholera toxin and cholera B-subunit as oral-mucosal adjuvant and antigen vector systems, *Vaccine* **11**:1179–1184.
- Hörnquist, E., *et al.*, 1994, Cholera toxin and cholera B-subunit as oral-mucosal adjuvant carrier systems, in: *Novel Delivery Systems for Oral Vaccines* (O'Hagan, ed.), CRC Press, Boca Raton, p. 153.
- Zhou, F., 1994, IgA-coated liposomes as a rectal vaccine delivery system for induction of secretory IgA in the rectal and colonic mucosa, Workshop on HIV/SIV Pathogenesis, NIH/DIADS March 14–17.

CONTACT(S): Nils Lycke, University of Göteborg, S-413 46 Göteborg, Sweden, Ph: 46-31-604936; Fax: 46-31-827-647. Also: Marian R. Neutra, Childrens Hospital/Enders 461, Boston, MA 02115, Ph: 617-735-6229; Fax: 617-730-0404.

COMPONENT/ADJUVANT NAME: CRL1005
OTHER NAME(S): Block Copolymer P1205
STRUCTURE: ABA block polymer with mean values of $x = 8$ and $y = 205$.



SOURCE: Linear chain polymers are synthesized by condensation of propylene oxide and ethylene glycol initiator in the presence of a cesium salt catalyst to form polyoxypropylene chain, followed by condensation of ethylene oxide on either end of the chain. Individual polymeric species of triblock nonionic block copolymers result from controlled synthesis of chains with predetermined length.

USES: A component of adjuvant formulations. The formulation is customized for particular uses. The water-in-oil emulsion typically contains 80% saline, and 20% oil phase consisting of squalene and Span 80. The copolymer is added to the aqueous phase in amounts sufficient for the required dose. It acts as both an adjuvant and stabilizer. The water-in-oil-in-water (w/o/w) multiple emulsion is prepared similarly with the addition of an outer aqueous phase.

APPEARANCE: Clear, colorless to slightly yellow, viscous liquid.

MOLECULAR WEIGHT: Approx. 12,500.

RECOMMENDED STORAGE: CRL1005 can be stored in tight amber glass containers with minimum headspace at 4°C for 2–3 years. Aqueous solutions (<10% w/v) stored at 4°C.

CHEMICAL/PHYSICAL PROPERTIES: CRL1005 is soluble in neutral or near neutral (pH 5.5–8) aqueous buffers at temperatures <4°C up to 10% (w/v). Above 4°C CRL1005 coalesces and forms large, stable micellelike structures 250–300 nm in diameter.

INCOMPATIBILITY: CRL1005 is compatible with aqueous buffering systems and can incorporate into the oil phase of oil-based emulsion vehicles. Compatible with a wide number of antigens, but more effective with intact proteins than peptides.

SAFETY/TOXICITY: Aqueous polymer suspensions of CRL1005 evaluated in rodent species in conjunction with influenza HA vaccines, with no adverse safety events noted. Microemulsions of CRL1005 with squalene evaluated in rodent species, with no adverse effects. Data not yet available for human safety.

ADJUVANT PROPERTIES: CRL1005 forms microparticulate structures that can bind a variety of antigens via a combination of hydrophobic interactions and surface charge. Available data suggest block copolymers influence epitope recognition and induce protective (e.g., IgG2a) antibody subclasses. Some formulations, particularly multiple emulsions, have potential as mucosal delivery vehicles. Data on cellular immunity not available.

- Hunter, R. L., *et al.*, 1981. Studies on the adjuvant activity of nonionic block polymer surfactants. I. The role of hydrophile–lipophile balance. *J. Immunol.* **127**:1244–1250.
- Hunter, R. L., *et al.*, 1991. Adjuvant activity of nonionic block copolymers, IV. Effect of molecular weight and formulation on titer and isotype of antibody, *Vaccine* **9**:250–256.
- Takayama, K., *et al.*, 1991. Adjuvant activity of nonionic block copolymers, V. Modulation of antibody isotype by lipopolysaccharides, lipid A and precursors, *Vaccine* **9**:257–265.

- Kalish, M. L., *et al.*, 1991, Murine IgG isotype responses to the *Plasmodium cynomolgi* circumsporozoite protein (NAGG)5. I. Effects of carrier, copolymer adjuvants, and nontoxic LPS on isotype distribution, *Infect. Immun.* **59**:2750–2757.
- van de Wijgert, J. H. H. M., *et al.*, 1991, Immunogenicity of *Streptococcus pneumoniae* type 14 capsular polysaccharide: Influence of carrier and adjuvants on isotype distribution, *Infect. Immun.* **59**:2750–2757.
- ten Hagen, T. L. M., *et al.*, 1993, The role of adjuvants in the modulation of antibody specificity and induction of protection by whole blood-stage *Plasmodium yoelii* vaccines, *J. Immunol.* **151**:7077–7085.
- Brey, R. N., Chapter 11, this volume.

CONTACT(S): Dr. Mark Newman, Vaxcel Corporation, GA 30092, Ph: 404-447-9330; Fax: 404-447-8875.

COMPONENT/ADJUVANT NAME: Cytokine-Containing Liposomes

OTHER NAME(S): Cytokine-containing Dehydration Rehydration Vesicles.

STRUCTURE: This is a dehydration–rehydration liposome composed of phosphatidylcholine (PC) and cholesterol in a 1:1 molar ratio and recombinant cytokines. The following cytokines have been tested: IL-1α, IL-1β, IL-6, TNFα, and interferon-γ.

SOURCE: The lipids are purchased from Avanti Polar-Lipids, Inc., Alabaster, AL. Cytokines are purchased from commercial sources and should not be contaminated with endotoxin.

USES: Induces both cellular and humoral immunity.

APPEARANCE: Cloudy suspension when in solution or white powder when dried.

MOLECULAR WEIGHT: See below for physical properties.

RECOMMENDED STORAGE: Prepared immediately before use, but may be stored at 4°C.

CHEMICAL/PHYSICAL PROPERTIES: The size of the liposomes has been determined to be between 1 and 8 μm by electron microscopy.

INCOMPATIBILITY: None known.

SAFETY/TOXICITY: Multilamellar liposomes are in clinical trials in humans.

ADJUVANT PROPERTIES:

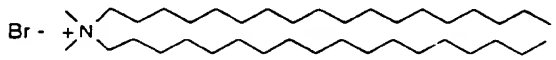
- Murray, J. L., *et al.*, 1989, Phase I trial of liposomal muramyl tripeptide phosphatidylethanolamine in cancer patients, *J. Clin. Oncol.* 7:1915–1925.
- Fidler, I. J., 1988, Targeting of immunomodulators to mononuclear phagocytes for therapy of cancer, *Adv. Drug Deliv. Res.* 2:69–83.
- Fogler, W. E., *et al.*, 1985, Distribution and fate of free and liposome-encapsulated [^{3}H]nor-muramyl dipeptide and [^{3}H]muramyl tripeptide phosphatidylethanolamine in mice, *J. Immunol.* **135**:1372–1377.
- Lopez Berestein, G., *et al.*, 1985, Liposomal amphotericin B for the treatment of systemic fungal infections in patients with cancer: A preliminary study, *J. Infect. Dis.* **151**:704–710.
- Gregoriadis, G., *et al.*, 1987, Liposomes as immunological adjuvants: Antigen incorporation studies, *Vaccine* 5:145–151.
- Lachman, L., *et al.*, Chapter 29, this volume.

CONTACT(S): Lawrence B. Lachman, Ph.D., University of Texas M. D. Anderson Cancer Center, Department of Cell Biology, Houston, TX 77030, Ph: 713-792-8587; Fax: 713-797-9764; E-mail: AN10010@MDACC.MDA.UTH.TMC.EDU.

COMPONENT/ADJUVANT NAME: DDA

OTHER NAME(S): Dimethyldioctadecylammonium bromide; dimethyldistearylammonium bromide (CAS Registry Number 3700-67-2).

STRUCTURE:



SOURCE: The chloride analogue of DDA is present in materials known as di(hydrogenate tallow)dimethylammonium salts available under various trade names (e.g., Quarternium-18, Adogen 442-110P, Cycloton D261 C/75). These salts comprise alkyl chains ranging from 12 to 18 carbon atoms with a typical distribution of C12:C14:C16:C18 = 1:4:31:64.

USES: For stimulation of immune responses against various antigens and especially delayed-type hypersensitivity. Oil-based emulsions in association with liposomes; also as a nonoil emulsion.

APPEARANCE: White, odorless powder.

MOLECULAR WEIGHT: 631

RECOMMENDED STORAGE: 4–20°C. Protect from light.

CHEMICAL/PHYSICAL PROPERTIES: Hydrophilic quaternary amine; positively charged surface-active substance with bromide (optionally chloride) as counterion. Gel–liquid transition temperature of 39.5°C. Poorly soluble in cold water but readily soluble/dispersible in warm water in which it forms liposomal structures. Soluble in organic solvents.

INCOMPATIBILITY: Complexes are formed with multivalent, negatively charged molecules (e.g., phosphate) in aqueous phase which might precipitate.

SAFETY/TOXICITY: Parenteral administration of DDA induces a mild inflammatory reaction at the site of injection (swelling and influx of polymorphonuclear neutrophils, macrophages, and lymphocytes). Effective dose range 1–10 mg/kg in small animals, and 0.01–1 mg/kg in large animals. Human trials include:

- Stanfield, J. P., *et al.*, 1973, Single dose antenatal tetanus immunization. *Lancet* **301**:215–219.
- Chambers, J. D., *et al.*, 1980, Induction of specific transplantation tolerance in man by autoblast immunization. *Blood* **41**:229–236.

ADJUVANT PROPERTIES: DDA stimulates both humoral and cell-mediated immune responses against a wide range of antigens and in various animal species. Especially delayed-type hypersensitivity reactions are augmented strongly by DDA after administration via subcutaneous for intracutaneous route. Functions as a carrier of antigen by direct binding of antigen, or modification at the oil/water interface.

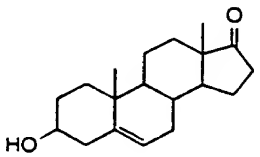
- Hilgers, L. A. T., and Snippe, H., 1992, DDA as immunological adjuvant. *Res. Immunol.* **143**:494–503.
- Snippe, H., and Kraayeveld, C., 1989, The immunoadjuvant dimethyldioctadecylammonium bromide, in: *Immunological Adjuvants and Vaccines* (G. Gregoriadis, A. C. Allison, and G. Poste, eds.), Plenum Press, New York, pp. 47–59.

CONTACT(S): L. Hilgers, Solvay S.A., Research & Technology, Central Laboratory, Applied Immunology, Rue de Ransbeek 310, B-1120 Brussels, Belgium, or H. Snippe, University Utrecht, Eijkman-Winkler Laboratorium for Medical Microbiology, 3584 CX Utrecht, Netherlands. Also: Eastman Kodak Company, Rochester, NY 14650, Ph: 716-458-3702; Fax: 716-722-3172. Also: Huw Hughes, M6 Pharmaceuticals, Inc., New York, NY 10701, Ph: 212-308-7200 ext. 14; E-mail: 74577.345@compuserve.com.

COMPONENT/ADJUVANT NAME: DHEA

OTHER NAME(S): Dehydroepiandrosterone; 5-androsten-3β-ol-17-one; dehydroisoandrosterone; androstenolone; prasterone; transdehydroandrosterone; DHA

STRUCTURE:



SOURCE: Commercially available from numerous suppliers. For citations involving the initial identification and methods of preparation see the *Merck Index* (Merck and Co., Inc., Rahway, NJ) under the entry for prasterone.

USES: DHEA can be directly incorporated into vaccine formulations (2–10 μg/vaccination in mice, 100 μg/vaccination in dogs) and will enhance antibody formation.

APPEARANCE: White, colorless powder or crystals.

MOLECULAR WEIGHT: 288.4

RECOMMENDED STORAGE: DHEA is relatively stable; however, its double bond is susceptible to both addition and oxidation reactions. Long-term storage under dry inert gas at or below –20°C.

CHEMICAL/PHYSICAL PROPERTIES: DHEA is a hydrophobic molecule with limited solubility in water. It is soluble in organic solvents including alcohols and dimethylsulfoxide, and only slightly soluble in petroleum ether. Literature melting point varies: dimorphous needles 140–141°C, leaflets 152–153°C (*Merck Index*), crystals 149–151°C (Steraloids, Wilton, NH).

INCOMPATIBILITY: Do not combine DHEA with components capable of addition or oxidation reactions involving the *p* electron system, including components capable of releasing free halogens.

SAFETY/TOXICITY: Dosages up to 1600 mg per day have been given orally to humans with no adverse reaction.

ADJUVANT PROPERTIES: Administration of DHEA or its sulfate to animals that are immunologically compromised as a consequence of age rapidly restore normal immunologic competence. DHEA can be administered systemically (approximately 4 mg/kg/day) to animals at the time of vaccination, or can be directly incorporated into the vaccine formulation.

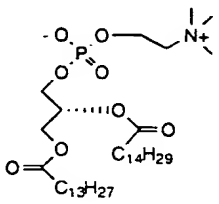
- Araneo, B. A., *et al.*, 1993, Reversal of the immunosenescent phenotype by dehydroepiandrosterone: Hormone treatment provides an adjuvant effect on the immunization of aged mice with recombinant hepatitis B surface antigen, *J. Infect. Dis.* **167**:830–840.
- Daynes, R. A., and Araneo, B. A., 1992, Prevention and reversal of some age-associated changes in immunologic responses by supplemental dehydroepiandrosterone sulfate therapy. Aging: *Immunol. Infect. Dis.* 3:135–154.
- Araneo, B. A., *et al.*, 1993, Administration of DHEA to burned mice preserves normal immunologic competence, *Arch. Surg.* **128**:318–325.

CONTACT(S): Raymond A. Daynes, Department of Pathology, University of Utah Medical Center, Salt Lake City, UT 84132, Ph: 801-581-3013; Fax: 801-581-8946.

COMPONENT/ADJUVANT NAME: DMPC

OTHER NAME(S): Dimyristoyl phosphatidylcholine; sn-3-phosphatidylcholine-1, 2-dimyristoyl; 1, 2-dimyristoyl-sn-3-phosphatidylcholine (CAS Registry Number 18194-24-6).

STRUCTURE: $C_{36}H_{72}NO_8P$



SOURCE: Chemical synthesis.

- Walts, A. E., *et al.*, 1992, Applications of biocatalysts in the synthesis of phospholipids, in: *Chirality in Industry* (A. N. Collins, G. N. Sheldrake, and J. Crosby, eds.), Wiley, New York.

USES: Used in the manufacture of pharmaceutical-grade liposomes, typically in combination with DMPG and/or cholesterol. Also used in adjuvant systems for vaccine formulations. Applications in novel forms of drug delivery.

APPEARANCE: White powder.

MOLECULAR WEIGHT: 677.9

RECOMMENDED STORAGE: Keep airtight. Keep out of light. Store at 0–5°C.

CHEMICAL/PHYSICAL PROPERTIES: Amphiphilic solid. Poorly soluble in water. Solubility tends to increase as the pH is lowered within the range pH 3–8. Subject to hydrolysis below pH 3 and above pH 8.

INCOMPATIBILITY: Avoid strong bases.

SAFETY/TOXICITY: Has been used in numerous clinical trials without reported safety/toxicity issues.

ADJUVANT PROPERTIES:

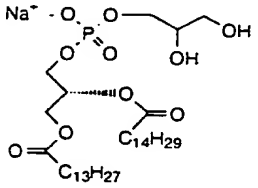
- Alving, C. R., 1993, Immunologic presentation of liposomal antigens, *J. Liposome Res.* **3**:493–504.
- Just, M., *et al.*, 1992, A single vaccination with an inactivated hepatitis A liposome vaccine induces protective antibodies after only two weeks, *Vaccine* **10**:737–739.
- Glück, R., Chapter 13, this volume.
- Pietrobon, P. J. F., Chapter 14, this volume.

CONTACT(S): Tony Newton, Genzyme Pharmaceuticals and Fine Chemicals, Cambridge, MA 02139, Ph: 617-252-7783; Fax: 617-252-7772.

COMPONENT/ADJUVANT NAME: DMPG

OTHER NAME(S): Dimyristoyl phosphatidylglycerol; sn-3-phosphatidylglycerol-1, 2-dimyristoyl, sodium salt (CAS Registry Number 67232-80-8); 1, 2-dimyristoyl-sn-3-phosphatidylglycerol

STRUCTURE: $C_{34}H_{66}O_{10}PNa$



SOURCE: Chemical synthesis.

- Walts, A. E., *et al.*, 1992, Applications of biocatalysts in the synthesis of phospholipids, in: *Chirality in Industry* (A. N. Collins, G. N. Sheldrake, and J. Crosby, eds.), Wiley, New York.

USES: Used in the manufacture of pharmaceutical-grade liposomes, typically in combination with DMPC and/or cholesterol. Also used in adjuvant systems for vaccine formulations. Applications in novel forms of drug delivery.

APPEARANCE: White powder.

MOLECULAR WEIGHT: 688.9

RECOMMENDED STORAGE: Keep airtight. Keep out of light. Store at 0–5°C.

CHEMICAL/PHYSICAL PROPERTIES: Amphiphilic solid. Poorly soluble in water. Solubility tends to increase as the pH is lowered within the range pH 3–8. Subject to hydrolysis below pH 3 and above pH 8.

INCOMPATIBILITY: Avoid strong bases.

SAFETY/TOXICITY: Has been used in numerous clinical trials without reported safety/toxicity issues.

ADJUVANT PROPERTIES:

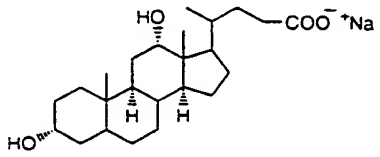
- Alving, C. R., 1993, Immunologic presentation of liposomal antigens, *J. Liposome Res.* **3**:493–504.
- Just, M., *et al.*, 1992, A single vaccination with an inactivated hepatitis A liposome vaccine induces protective antibodies after only two weeks, *Vaccine* **10**:737–739.
- Glück, R., Chapter 13, this volume.
- Pietrobon, P. J. F., Chapter 14, this volume.

CONTACT(S): Tony Newton, Genzyme Pharmaceuticals and Fine Chemicals, Cambridge, MA 02139, Ph: 617-252-7783; Fax: 617-252-7772.

COMPONENT/ADJUVANT NAME: DOC/Alum Complex

OTHER NAME(S): Deoxycholic Acid Sodium Salt; DOC/Al(OH)3/mineral carrier complex

STRUCTURE:



DOC

SOURCE: DOC obtained from Sigma Chemicals.

USES: DOC has been used as a detergent. Complex used as adjuvant formulation.

APPEARANCE: White powder, or a clear, colorless solution.

MOLECULAR WEIGHT: 414.6

RECOMMENDED STORAGE: 4°C.

CHEMICAL/PHYSICAL PROPERTIES: Gives typical reactions common for all bile acids. Optical rotation $[\alpha]_D 20 = +44 \pm 2°$ [c = 2% (w/v) in water].

INCOMPATIBILITY: Precipitates below pH 6 and in presence of divalent cations, forms gels at temperatures below 10°C (reversible).

SAFETY/TOXICITY: The DOC/Alum complex is non-toxic at adjuvant active doses, and has been used in humans without adverse side-effects.

- Nagy, L. K., 1972, The effect of deoxycholate on cholera vaccine, *Prog. Immunobiol. Stand.* 5:341–347.
- Mussgay, M. and Weiland, E., 1973, Preparation of inactivated vaccines against alphaviruses using Semiliki Forest virus–white mouse as a model. In Inactivation experiments and evaluation of double inactivated subunit vaccines, *Intervirology* 1:259–268.

ADJUVANT PROPERTIES: Enhances immune response to membrane proteins.

- Barrett, N., *et al.*, 1989, Large-scale production and purification of a vaccinia recombinant-derived HIV-1 gp160 and analysis of its immunogenicity, *AIDS Res. Hum. Retroviruses* 5:157–171.

CONTACT(S): Professor Friedrich Dorner, Immuno AG, Biomedical Research Center, A-2304 Ortha/Donau, Austria, Ph: 43-2212-2701/ext. 300; Fax: 43-2212-2716.

COMPONENT/ADJUVANT NAME: Freund's Complete Adjuvant

OTHER NAME(S): Complete Freund's adjuvant; CFA; FCA

STRUCTURE: Mixture of mineral oil (Marco 52) and emulsifier (Arlacel A [mannide monooleate]) as an emulsion of 85% mineral oil and 15% emulsifier with 500 μg heat-killed and dried *Mycobacterium tuberculosis* per mL of emulsifier mixture.

SOURCE: *M. tuberculosis* grown and adjuvant manufactured at the Statens Seruminstitut, Copenhagen, Denmark.

USES: The ethics of using Freund's complete adjuvant in animals is at present disputed, because of the profile of severe side effects.

APPEARANCE: Thick viscous liquid without color.

MOLECULAR WEIGHT: Not applicable.

RECOMMENDED STORAGE: Store at 2–8°C. Do not freeze the final emulsion, as it is disrupted by freezing.

CHEMICAL/PHYSICAL PROPERTIES: Mixing (usually syringe-to-syringe mixing) with an aqueous antigen phase in a 1:1 ratio makes a water-in-oil emulsion (w/o) ready for immunization.

INCOMPATIBILITY: Avoid freezing the final emulsion.

SAFETY/TOXICITY: May cause granulomas and abscesses at the site of injection. May cause arthritis, amyloidosis, and allergic reactions. Can cause ascites production in BALB/c mice when injected i.p. with or without antigen.

- Yamanaka, M., *et al.*, 1992, Pathological studies on local tissue reactions in guinea pigs and rats caused by four different adjuvants, *J. Vet. Med. Sci.* **54**:685–692.

ADJUVANT PROPERTIES:

- Freund, J., 1956, The mode of action of immunologic adjuvants, *Adv. Tuberc. Res.* **7**:130–148.
- Herbert, W. J., 1967, Methods for the preparation of water-in-oil, and multiple, emulsions for use as antigen adjuvants; and notes on their use in immunization procedures, in: *Handbook of Experimental Immunology* (D. M. Weir, ed.), Blackwell Scientific Publications, 1207–1214.
- Bomford, R., 1980, The comparative selectivity of adjuvants for humoral and cell-mediated immunity. II. Effect on delayed-type hypersensitivity in the mouse and guinea pig, and cell-mediated immunity to tumor antigens in the mouse of Freund's incomplete and complete adjuvants, Alhydrogel, *Corynebacterium parvum*, *Bordetella pertussis*, muramyl dipeptide and saponin, *Clin. Exp. Immunol.* **39**:435–441.

CONTACT(S): There are several contacts for CFA (see also Montanide ISA monographs). This product is by: Erik B. Lindblad, Superfos Biosector, DK-2950 Vedbaek, Denmark, Ph: 45 42 89 31 11; Fax: 45 42 89 15 95. Also: Al Reisch, Sergeant, Inc., Clifton, NJ 07012, Ph: 201-472-9111; Fax: 201-472-5686.

COMPONENT/ADJUVANT NAME: Freund's Incomplete Adjuvant

OTHER NAME(S): Incomplete Freund's Adjuvant; IFA; FIA

STRUCTURE: Mixture of mineral oil (Marcol 52) and emulsifier (Arlacel A [mannide monooleate]) as an 85% mineral oil and 15% emulsifier emulsion.

SOURCE: Manufactured by Statens Seruminstitut, Copenhagen, Denmark.

USES: Immunization of experimental animals.

APPEARANCE: Thick viscous liquid without color.

MOLECULAR WEIGHT: Not applicable.

RECOMMENDED STORAGE: Store at 2–8°C. Do not freeze the final emulsion, as it is disrupted by freezing.

CHEMICAL/PHYSICAL PROPERTIES: Mixing (usually syringe-to-syringe mixing) with an aqueous antigen phase in a 1:1 ratio makes a water-in-oil emulsion ready for immunization.

INCOMPATIBILITY: Avoid freezing the final emulsion.

SAFETY/TOXICITY: May cause granulomas and abscesses at the site of injection. Induces production of ascites in BALB/c mice when injected i.p. with or without antigen.

- Yamanaka, M., *et al.*, 1992, Pathological studies on local tissue reactions in guinea pigs and rats caused by four different adjuvants. *J. Vet. Med. Sci.* 54:685–692.

ADJUVANT PROPERTIES:

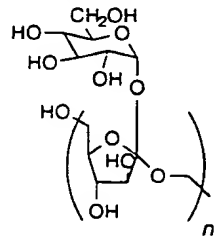
- Herbert, W. J., 1967, Methods for the preparation of water-in-oil, and multiple, emulsions for use as antigen adjuvants; and notes on their use in immunization procedures. in: *Handbook of Experimental Immunology* (D. M. Weir, ed.), Blackwell Scientific Publications, 1207–1214.
- Bomford, R., 1980, The comparative selectivity of adjuvants for humoral and cell-mediated immunity. II. Effect on delayed-type hypersensitivity in the mouse and guinea pig, and cell-mediated immunity to tumor antigens in the mouse of Freund's incomplete and complete adjuvants, Alhydrogel, *Corynebacterium parvum*, *Bordetella pertussis*, muramyl dipeptide and saponin. *Clin. Exp. Immunol.* **39**:435–441.

CONTACT(S): There are several contacts for IFA (see also Montanide ISA monographs). This product is by: Erik B. Lindblad, Superfos Biosector, DK-2950 Vedbaek, Denmark, Ph: 45 42 89 31 11; Fax: 45 42 89 15 95. Also: Al Reisch, Sergeant, Inc., Clifton, NJ 07012, Ph: 201-472-9111; Fax: 201-472-5686.

COMPONENT/ADJUVANT NAME: Gamma Inulin

OTHER NAME(S): None

STRUCTURE: Linear (unbranched) β-D-(2→1) polyfructofuranosyl-α-D-glucose, as particles in the gamma polymorphic configuration. Typically n = 50–75.



SOURCE: Dahlia tubers. Obtained by aqueous extraction and crystallization of inulin, followed by adsorptive treatments, recrystallization, and conversion to the gamma form at 37°C.

- Cooper, P. D., and Carter, M., 1986. Anticomplementary action of polymorphic 'solubility forms' of particulate inulin, *Mol. Immunol.* **23**:895–901.

USES: Highly specific activator of the alternative pathway of complement in vitro and in vivo. Included in adjuvant formulations as a primary adjuvant and also as the immune stimulant when combined as composite particles with alum in the adjuvant Algammulin.

APPEARANCE: Milky white, nonviscous aqueous suspension, easily resuspended. Supplied at 50 mg/mL, sterile and pyrogen-free.

MOLECULAR WEIGHT: 8000–12,000. A typical preparation comprises a range of chain lengths corresponding to degrees of polymerization of 50–75 fructose residues.

RECOMMENDED STORAGE: 2–8°C in aqueous medium. Do not freeze or heat over 45°C.

CHEMICAL/PHYSICAL PROPERTIES: Neutral, edible polysaccharide of known primary structure, as ovoids about 1 μm diameter. Stable for years under recommended storage. Unstable below pH 6 and above pH 10. The gamma form is virtually insoluble at 37°C and is essential for biological activity.

INCOMPATIBILITY: Degraded in moderately strong acid.

SAFETY/TOXICITY: Nonpyrogenic, nonantigenic, and of very low toxicity in experimental animals. Biodegradable to simple sugars. Large intravenous doses can cause acute complement-activation shock similar to that sometimes found in human renal dialysis patients. Dissolved inulin is pharmacologically inert and is registered for human use.

ADJUVANT PROPERTIES: Expected to stimulate immune responses by causing ligation of leukocyte-surface complement receptors (CR) via known biochemical mechanisms. Addition of gamma inulin is known to enhance both humoral and cell-mediated immunity from mainly Th1 pathways. Gamma inulin also has an antitumor action and an effect on natural immunity.

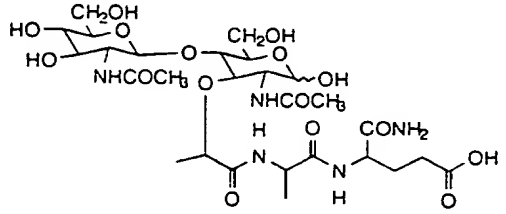
- Cooper, P. D., and Steele, E. J., 1988. The adjuvanticity of gamma inulin, *Immunol. Cell Biol.* **66**:345–352.
- Cooper, P. D., *et al.*, 1993. Gamma inulin and Algammulin: Two new vaccine adjuvants, in: *Vaccines 93, Modern Approaches to New Vaccines Including Prevention of AIDS* (H. S. Ginsburg, F. Brown, R. M. Chanock, and R. A. Lerner, eds.), Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, pp. 25–30.
- Cooper, P. D., Chapter 24, this volume.

CONTACT(S): Dr. Peter D. Cooper, Division of Cell Biology, John Curtin School of Medical Research, Australian National University, Canberra, A. C. T., Australia 2601, Ph: 61-6-291-8670; Fax: 61-6-249-2595.

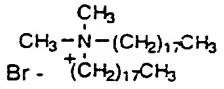
COMPONENT/ADJUVANT NAME: Gerbu Adjuvant

OTHER NAME(S): None

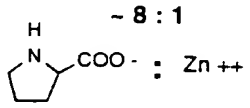
STRUCTURE: Mixture of: (1) *N*-acetylglucosaminyl-(β1-4)-*N*-acetylmuramyl-L-alanyl-D-glutamine (GMDP), (2) dimethyl dioctadecylammonium chloride (DDA), (3) zinc L-proline salt complex (Zn-Pro$_{-8}$) (shown below).



GMDP



DDA



Proline-Zinc (8:1)

SOURCE: (1) Semisynthetic, (2) synthetic, (3) semisynthetic.

USES: Proprietary adjuvant formulation intended for animal and human use.

APPEARANCE: White lyophilizate.

MOLECULAR WEIGHT: (1) GMDP = 695, (2) DDA = 631, (3) Pro$_{-8}$:Zn complex = ~1000.

RECOMMENDED STORAGE: Store at 2–8°C.

CHEMICAL/PHYSICAL PROPERTIES: GMDP is a crystalline solid, easily dispersible in aqueous antigen solutions. DDA by itself is very sparsely soluble (2.5 mg/L) but in the zinc L-proline complex it is easily dispersible in water. At 37°C it remains in dispersion for at least 1 week. Frozen solutions can be thawed repeatedly and the dispersion of DDA remains stable. The material is soluble in 70% ethanol. The preparation is sterile and suitable for injection after reconstitution. It is kept in 5-mL glass vials with stoppers of butyl rubber. Once reconstituted, the storage must be in the frozen state to prevent microbial growth or "contamination."

INCOMPATIBILITY: None found.

SAFETY/TOXICITY: All components are extensively tested for oral and parenteral toxicity and found to be nontoxic in doses well above those recommended for immunization. Zinc and L-proline are widely used in infusions for a variety of human uses in doses larger as used in this adjuvant formula.

ADJUVANT PROPERTIES: Gerbu Adjuvant has already been tested in many applications, mainly with mice, hens, and rabbits.

- Gruhofer, N., 1994, An adjuvant based on GMDP with DDA and zinc–L-proline complex as synergists, *Immunology Lett.* (in press).

CONTACT(S): Dr. P. Cooper, CC Biotech Corporation, Poway, CA 92064, Ph: 619-451-9949; Fax: 619-487-8138; Dr. N. Grubhofer, Gerbu Biotecknik GmbH D69251 Gaiberg, Germany, Ph: 49 6223 47197; Fax 49 6223 47199.

COMPONENT/ADJUVANT NAME: GM-CSF

OTHER NAME(S): Granulocyte–macrophage colony stimulating factor; Sargramostim (yeast-derived rh-GM-CSF)

STRUCTURE: GM-CSF is a glycoprotein of 127 amino acids. Recombinant human GM-CSF is produced in yeast and it differs from the natural human GM-CSF by substitution of Leu for Arg at position 23.

- Walter, M. R., *et al.*, 1992, Three-dimensional structure of recombinant human granulocyte–macrophage colony stimulating factor, *J. Mol. Biol.* **224**:1075–1085.

Sequence of recombinant human GM-CSF (Sargramostin):

APARSPSPSTQPWEHVNAIQEALRLLNLSRDTAAEMNETVEVISEMFDLQEPTC
LQTRLELYKQGLRGSLTKLKGPLTMMASHYKQHCPPTPETSCATQIITFESFKE
NLKDFLLVIPFDCWEPVQE

SOURCE: Recombinant protein produced in yeast (*S. cerevisiae*).

USES: GM-CSF (Sargramostin) is an approved product indicated for acceleration of myeloid recovery in patients with non-Hodgkin's lymphoma, acute lymphoblastic leukemia, and Hodgkin's disease undergoing autologous bone marrow transplantation. Reports in the literature also suggest that GM-CSF is able to activate mature granulocytes and macrophages, and may have utility as co-adjuvant.

APPEARANCE: White, lyophilized powder (before reconstitution), or a clear colorless solution (after reconstitution).

MOLECULAR WEIGHT: 15,500, 16,800, and 19,500 (three bands on SDS-PAGE representing variation in glycosylation).

RECOMMENDED STORAGE: Both lyophilized GM-CSF (Sargramostin) and reconstituted product should be stored at 2–8°C. Lyophilized product may also be frozen at –20 or –70°C.

CHEMICAL/PHYSICAL PROPERTIES: GM-CSF (Sargramostin) exists as a major species (pI 5.2) and a minor species (pI 4.5–5.2). GM-CSF (Sargramostin) shows a specific activity of 5.6×10^6 IU/mg as measured in a TF-1 cell proliferation assay.

INCOMPATIBILITY: Avoid contact with proteases.

SAFETY/TOXICITY: Generally well tolerated when given for indications above. Safety data using GM-CSF as adjuvant have not been reported.

ADJUVANT PROPERTIES: This cytokine is a growth factor that stimulates normal myeloid precursors, and activates mature granulocytes and macrophages.

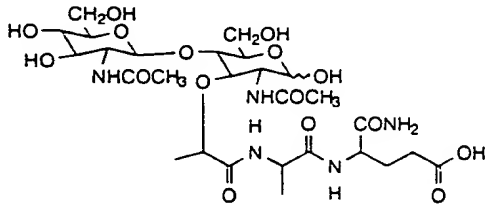
- Tao, M. H., and Levy, R., 1993, Idiotype/granulocyte–macrophage colony stimulating factor fusion protein as a vaccine for B-cell lymphoma, *Nature* **362**:755–758.
- Dranoff, G., *et al.*, 1993, Vaccination with irradiated tumor cells engineered to secrete murine granulocyte–macrophage colony stimulating factor stimulates potent, specific, and long-lasting anti-tumor immunity, *Proc. Natl. Acad. Sci. USA* **90**:3539–3543.

CONTACT(S): Dr. Michael Widmer, Immunex Corp., Seattle, WA 98101, Ph: 206-587-0430: Fax: 206-587-0606.

COMPONENT/ADJUVANT NAME: GMDP

OTHER NAME(S): *N*-Acetylglucosaminyl-(β1-4)-*N*-acetylmuramyl-L-alanyl-D-isoglutamine (CAS Registry Number 70280-03-4)

STRUCTURE:



SOURCE: Semisynthetic. Disaccharide isolated from microbial source, dipeptide wholly synthetic. U.S. Patent No. 4,395,399.

USES: Primary adjuvant.

APPEARANCE: White, lyophilized powder.

MOLECULAR WEIGHT: 695. Contains defined low percentages of acetate and water.

RECOMMENDED STORAGE: Extremely stable at room temperature under dry conditions. For prolonged storage, desiccator at 4°C is recommended.

CHEMICAL/PHYSICAL PROPERTIES: Mp 166–170°C; pKa 5.48; optical rotation $[\alpha]_D20 = +2.8°$. Exists as an equilibrium mixture of two anomeric forms due to mutarotation of the -OH at C-1 of the muramic acid residue. Highly soluble in aqueous buffers, ethanol, methanol, DMF. Practically insoluble in chloroform, ether, and acetonitrile.

INCOMPATIBILITY: Avoid extremes of pH.

SAFETY: Extensive Phase I systemic safety data in humans (mostly after oral administration). Single oral doses of up to 50 mg given with no side effects. Intramuscular injections of 1 mg given with minimal local reaction. LD_{50} in mouse = 7 g/kg. Less pyrogenic than prototype muramyl dipeptide. In Phase II clinicals for other applications.

ADJUVANT PROPERTIES: Highly effective primary adjuvant in a range of vehicles; aqueous buffers, mineral oil, pluronic/squalane/Tween emulsions. Also effective as oral adjuvant, enhancing mucosal IgA response.

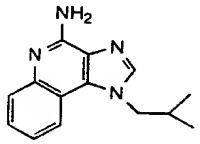
- Andronova, T. M., and Ivanov, V. T., 1991, The structure and immunomodulating function of glucosaminylmuramyl peptides, *Sov. Med. Rev. D Immunol.* **4**:1–63.
- Bomford, R., *et al.*, 1992, The control of the antibody isotype response to recombinant human immunodeficiency antigen by adjuvants, *AIDS Res. Hum. Retroviruses* **8**:1765–1771.
- Campbell, M. J., *et al.*, 1990, Idiotype vaccination against murine B-cell lymphoma, *J. Immunol.* **145**:1029–1036.

CONTACT(S): Philip Ledger, Ph.D., Peptech(*UK*) Ltd., Cirencester, Glos GL7 2PF, United Kingdom, Ph: 44-285-643666; Fax: 44-285-644328.

COMPONENT/ADJUVANT NAME: Imiquimod

OTHER NAME(S): 1-(2-methypropyl)-1*H*-imidazo[4,5-*c*]quinolin-4-amine; R-837; S-26308

STRUCTURE:



SOURCE: Chemical synthesis.

- Gerster, J. F., 1987, U.S. Patent 4,680,338. Lagain, D., 1991, U.S. Patent 4,988,815.

USES: Included in adjuvant formulations as a primary adjuvant component.

APPEARANCE: White, fine crystalline solid.

MOLECULAR WEIGHT: 240.31 free base, 276.77 hydrochloride salt.

RECOMMENDED STORAGE: Solid is stable at room temperature. Shelf life is acceptable.

CHEMICAL/PHYSICAL PROPERTIES: Very limited solubility as the free base. The hydrochloride salt is soluble in water at concentrations up to 10 mg/mL. The optimal solubility for use is pH ~4.

INCOMPATIBILITY: None found.

SAFETY/TOXICITY: Imiquimod's safety package includes extensive evaluation with 4-month dermal and 6-month oral studies completed. In addition, no teratogenic or mutagenic effects were seen. Phase III trials as a topical antiviral agent using 5% cream, and Phase IIA trials as an oral antiviral agent using 50 and 200 mg doses of drug are ongoing.

ADJUVANT PROPERTIES: Addition of imiquimod known to induce both humoral and cell-mediated immunity via induction of cytokines from monocytes and macrophages.

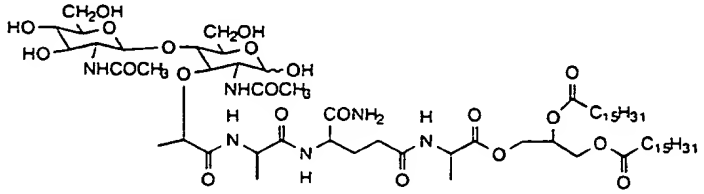
- Bernstein, D. L., *et al.*, 1993. Adjuvant effects of imiquimod on a herpes simplex virus type 2 glycoprotein vaccine in guinea pigs. *J. Infect. Dis.* **167**:731–735.
- Sidky, Y. A., *et al.*, 1992. Inhibition of murine tumor growth by an interferon-inducing imidazoquinolinamine. *Cancer Res.* **52**:3528–3533.
- Reiter, M. J., *et al.*, 1994. Cytokine induction in mice by the immunomodulator imiquimod. *J. Leukocyte Biol.* **55**:234–240.

CONTACT(S): R. C. Hanson, Business Development, 3M Pharmaceuticals, St. Paul, MN 55144, Ph: 612-737-3137; Fax: 612-737-4556.

COMPONENT/ADJUVANT NAME: ImmTher™

OTHER NAME(S): *N*-Acetylglucosaminyl-*N*-acetylmuramyl-L-Ala-D-isoGlu-L-Ala-glycerol dipalmitate; DTP-GDP

STRUCTURE:



SOURCE: Synthetic (U.S. Patent 4,950,645).

USES: ImmTher™ is a potent macrophage activator, capable of inducing remission in human metastatic colorectal cancer. In vitro and in vivo it induces high levels of TNF, IL-1, and IL-6. The active drug compound is formulated as adjuvant in liposomes consisting of 175 mg of 1-palmitoyl-2-oleoyl phosphatidylcholine and 75 mg of 1,2-dioleoyl phosphatidylglycerol (per 2.5 mL).

APPEARANCE: White, odorless powder. The lyophilized product is reconstituted in 2.5 mL saline to produce an initial concentration of 400 μg/mL.

MOLECULAR WEIGHT: 1316.82

RECOMMENDED STORAGE: Stable as a lyophilized powder or in solution with saline or PBS at 3–8°C for 5 years.

CHEMICAL/PHYSICAL PROPERTIES: Amphoteric molecule soluble in chloroform:methanol (7:3), and tert-butanol. The ester bond between the peptide and the lipid is subject to hydrolysis.

INCOMPATIBILITY: Avoid strong acids and bases.

SAFETY/TOXICITY: Safe in humans up to single doses of 1.2 mg/m^2 and given weekly for up to 6 months at doses of 0.8 to 1.0 mg/m^2. Major toxicity is fever, chills, and hypotension at doses of 0.8 mg/m^2 or greater. There has been no observed hematological, hepatic, or neural toxicity.

ADJUVANT PROPERTIES: The formulation is a potent macrophage activator and enhances both cellular and humoral immunity.

- Vosika, G. J., *et al.*, 1991. Phase I trial of ImmTher™, a new liposome-incorporated lipophilic disaccharide tripeptide, *J. Immunother.* **10**:256–266.
- Vosika, G. J., *et al.*, 1990. Immunologic and toxicologic study of disaccharide tripeptide glycerol dipalmitoyl: A new lipophilic immunomodulator. *Mol. Biother.* **2**:50–56.

CONTACT(S): Gerald Vosika, M. D., ImmunoTherapeutics, Inc., Fargo, ND 58104, Ph: 701-232-9575; Fax: 701-237-9275.

COMPONENT/ADJUVANT NAME: Interferon-γ

OTHER NAME(S): Actimmune® (rhIFN-gamma, Genentech, Inc.); immune interferon; IFN-γ; gamma-interferon

STRUCTURE: Noncovalent dimer. Low-resolution crystal structure available. Monomer consists of 140 amino acids, no glycosylation or cysteines in human form. Murine form is a covalent dimer (one cysteine per monomer).

- Ealick, S. E., *et al.*, 1991, Three-dimensional structure of recombinant human interferon-γ, *Science* **252**:698–702.

Sequence of human interferon-gamma:

QDPYVKEAENLKKYFNAGHSDVADNGTLFLGILKNWKEESDRKIMQSQIVSF
YFKLFKNFKDDQSIQKSVETIKEDMNVKFFNSNKKKRDDFEKLTNYSVTDLN
VQRKAIHELIQVMAELSPAAKTGKRKRSQMLFRGRRASQ

SOURCE: Both human (rhIFN-gamma) and murine (rmuIFN-gamma) forms are expressed in *Escherichia coli* and distributed in a completely pure state.

USES: rhIFN-gamma (Actimmune®) is FDA-approved for use in chronic granulomatous disease (CGD). Currently, Actimmune® is in human clinical Phase III trials for renal cell carcinoma. rhIFN-gamma has been studied in humans as an adjuvant for hepatitis B subunit antigen (Quiroga *et al.*, 1990, *Hepatology* **12**:661–663).

APPEARANCE: Clear aqueous solution.

MOLECULAR WEIGHT: Monomer 16,440

RECOMMENDED STORAGE: 2–8°C (do not freeze).

CHEMICAL/PHYSICAL PROPERTIES: rhIFN-gamma: pI 9.9, absorptivity = 0.75 $(mg/mL)^{-1}$ cm^{-1} at 280.4 nm, typical specific activity ~3–5 × 10^7 IU/mg. rmuIFN-gamma: absorptivity = 0.93 $(mg/mL)^{-1}$ cm^{-1} at 280 nm, typical specific activity ~0.5–1 × 10^7 IU/mg.

INCOMPATIBILITY: Susceptible to shear-induced degradation (requires surfactants for stability), readily deamidates at high pH (> 6.5), and may be cleaved by proteases.

SAFETY/TOXICITY: rhIFN-gamma as Actimmune® is an FDA-approved commercial product for human use. Standard human dose is 100 μg. High doses can cause significant side effects such as nausea, fever, and other flulike symptoms. Effect of molecule is specific to species. Human form does not elicit toxicity in lower species at several mg/kg doses which are toxic to humans.

ADJUVANT PROPERTIES: Higher and earlier neutralizing antibody titers, increase in duration of neutralizing antibody titers, increase in MHC class II expression on antigen-presenting cells, increase in helper T cell levels, and an improved DTH response have all been observed when IFN-gamma was administered with an antigen. The IFN-gamma must be given at the same site and at the same time (within 6 h) as the antigen to have biological effect.

- Schijns, V. E. C. J., *et al.*, 1994, Modulation of antiviral immune responses by exogenous cytokines: Effects of tumour necrosis factor-α, interleukin-1α, interleukin-2 and interferon-γ on the immunogenicity of an inactivated rabies vaccine, *J. Gen. Virol.* **75**:55–63.
- Heath, A. W., and Playfair, J. H. L., 1992, Cytokines as immunological adjuvants, *Vaccine* **10**:427–434.
- Cao, M., *et al.*, 1992, Enhancement of the protective effect of inactivated influenza virus vaccine by cytokines, *Vaccine* **10**:238–242.
- Heath, A., Chapter 28, this volume.
- Dong, P., *et al.*, Chapter 27, this volume.

CONTACT(S): Dr. Jeffrey L. Cleland, Genentech, Inc., South San Francisco, CA 94080. Ph: 415-225-3921; Fax: 415-225-3979; E-mail: Cleland.Jeffrey@gene.com.

COMPONENT/ADJUVANT NAME: Interleukin-1β

OTHER NAME(S): IL-1β; IL-1; human interleukin-1β mature polypeptide 117-259

STRUCTURE: This protein is composed of 12 antiparallel β-strands folded into a six-stranded β barrel, with 3-fold symmetry about the axis of the barrel.

- Priestle, J. P., *et al.*, 1988, Crystal structure of the cytokine interleukin-1β, *EMBO J.* 7:339–343.

Sequence of ILβ:

APVRSLNCTLRDSQQKSLVMSGPYELKALHLQGQDMEQQVVFSMSFVQGEES
NDKIPVALGLKEKNLYLSCVLKDDKPTLQLESVDPKNYPKKKMEKRFVFNKIE
INNKLEFESAQFPNWYISTSQAENMPVFLGGTKGGQDITDFTMQFVSS

SOURCE: Recombinant mature fragment 117–259 of human interleukin-1β, usually expressed in *E. coli* or other bacteria, derived from myeloid or placental libraries. Purified by sequential steps or ion-exchange chromatography and gel filtration.

USES: Primary adjuvant. Active by oral, intravenous, intraperitoneal, and subcutaneous routes. It can be administered admixed with antigen or separately.

APPEARANCE: White, odorless powder.

MOLECULAR WEIGHT: 17,377

RECOMMENDED STORAGE: Store lyophilized powder dry at –20 or 4°C. The concentrated solution must be aliquoted and stored at –80°C. Avoid freeze-thawing, which results in rapid loss of activity. If diluted in solution, it tends to adhere to the vessel walls: use siliconized glass, high protein concentrations, low temperature, stabilizing proteins.

CHEMICAL/PHYSICAL PROPERTIES: The recombinant protein is quite unstable: it does not stand storage in solution at 4°C nor does it stand freeze-thawing. The protein contains unreduced cysteines which form interchain disulfide bridges on prolonged storage (both in frozen and lyophilized conditions), with the appearance of multimeric molecules devoid of biological activity. pI 6.9.

INCOMPATIBILITY: Avoid proteases.

SAFETY/TOXICITY: IL-1 is a major inflammatory mediator; thus its use in vivo may have many unwanted effects. Phase I trials demonstrated severe hypotension as major side effect, as well as pain, respiratory and hematological alterations. However, the immunostimulatory effects of IL-1 are evident at doses much lower than those yielding toxicity.

ADJUVANT PROPERTIES: It increases both T-dependent and T-independent responses to different types of antigens. Active on both primary and secondary responses.

- Staruch, M. J., and Wood, D. D., 1983, The adjuvanticity of interleukin-1 in vivo, *J. Immunol.* **130**:2191–2194.
- Nencioni, L., *et al.*, 1987, In vivo immunostimulating activity of the 163–171 peptide of human IL-1β, *J. Immunol.* **139**:800–804.
- Frasca, D., *et al.*, 1988, In vivo restoration of T cell functions by human IL-1β or its 163–171 nonapeptide in immunodepressed mice, *J. Immunol.* **141**:2651–2655.
- McCune, C. S., and Marquis, D. M., 1990, Interleukin-1 as an adjuvant for active specific immunotherapy in a murine tumor model, *Cancer Res.* **50**:1212–1215.
- Heath, A., Chapter 28, this volume.
- Dong, P., *et al.*, Chapter 27, this volume.

CONTACT(S): Dr. Diana Boraschi, Dompè Research Center, Via Campo di Pile, 1-67100 L'Aquila, Italy, Ph: 39-862-338324; Fax: 39-862-338219.

COMPONENT/ ADJUVANT NAME: Interleukin-2

OTHER NAMES: IL-2; T-cell growth factor; aldesleukin (des-alanyl-1, serine-125 human interleukin-2); Proleukin®; Teceleukin®

STRUCTURE: Native human IL-2 contains 133 amino acids (see below); aldesleukin contains 132 amino acids. IL-2 exists as six alpha-helical domains, termed A to F. Glycosylation not essential for function.

- Rosenberg, S. A., *et al.*, 1983, Biological activity of recombinant human interleukin-2 produced in *Escherichia coli*, *Science* **223**:1412–1414.
- Brandhuber, B. J., *et al.*, 1987, Three dimensional structure of interleukin-2, *Science* **238**:1707–1709.
- Ju, G., *et al.*, 1987, Structure function analysis of human interleukin-2: Identification of amino acid residues required for biological activity, *J. Biol. Chem.* **262**:5723–5731.

Sequence of human IL-2:

APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKATEL
KHLQCLEEELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEY
ADETATIVEFLNRWITFCQSIISTLT

SOURCE: Recombinant protein expressed in *E. coli*.

USES: As a primary adjuvant, co-emulsified with antigens and lipids, with polyethylene glycol-modified long-acting form (PEG IL-2), or liposome-encapsulated sustained release dosage form. Aldesleukin (Proleukin®) is an FDA-licensed agent for treatment of metastatic renal cell carcinoma.

APPEARANCE: Lyophilized, white to off-white solid; reconstituted with water for injection to give a clear, colorless solution.

MOLECULAR WEIGHT: 15,300

RECOMMENDED STORAGE: Store lyophilized aldesleukin solid at 2–8°C. Store the reconstituted product at 2–8°C for no longer than 48 h. Reconstituted solution diluted with 5% dextrose to a 200 μg/mL IL-2 concentration stable in plastic syringes at 2–8°C for 14 days. Store lyophilized PEG IL-2 at –20°C and reconstituted PEG IL-2 at 2–8°C for up to 28 days. Storage stability of liposomal dosage form unknown.

CHEMICAL/ PHYSICAL PROPERTIES: Relatively hydrophobic protein with moderate aqueous solubility (~1 mg/mL). Major pI = 8.0. Adsorbs to glass and plastic surfaces below concentrations of 10 μg/mL or less, this can be prevented by having 0.1% human albumin present in the diluting solution prior to adding aldesleukin. Potential degradation pathways: methionine oxidation, aggregation, dimer and higher oligomer formation and deamidation.

INCOMPATIBILITY: Aldesleukin is not compatible with sodium chloride for injection, solutions with high ionic strength or containing preservatives. May degrade with proteases; avoid solutions of low or high pH extremes. Compatible with mineral oil and other lipoidal adjuvants (DDA, Avridine®).

SAFETY/TOXICITY: Frequency and severity of adverse reactions are generally dose-related. The most frequently reported serious adverse reactions include hypotension, renal dysfunction, dyspnea, and mental-state changes. Further descriptions are indicated in the Proleukin® (Aldesleukin for Injection) package insert (Chiron Corporation, Emeryville, CA).

- Rosenberg, S. A., *et al.*, 1985, Observations on the systemic administration of autologous lymphokine-activated killer cells and recombinant interleukin-2 to patients with metastatic cancer, *N. Engl. J. Med.* **313**:1485–1492.

- Kroemer, G., *et al.*, 1992, Interleukin-2, a pro-autoimmune lymphokine that interferes with post-deletional tolerance, *Semin. Immunol.* **4**:167–179.

ADJUVANT PROPERTIES: IL-2 supports the growth and proliferation of antigen-activated T lymphocytes and plays a central role in the cascade of cellular events involved in the immune response. Proliferating T cells also produce a variety of other lymphokines which may modulate other arms of the immune system. In view of these direct and indirect actions of IL-2 on the immune response, IL-2 may function as an adjuvant to vaccination by increasing the specific and durable response to vaccine immunogens. Low doses may give up to 25-fold increase in adjuvant effect, with inhibition of adjuvant effect at high doses. May induce cellular immunity when given systemically, and IgA when administered at a mucosal surface.

- Weinberg, A., and Merrigan, T. C., 1988, Recombinant interleukin-2 as an adjuvant for vaccine-induced protection, *J. Immunol.* **140**:294–299.
- Nunberg, J. H., *et al.*, 1989, Interleukin 2 acts as an adjuvant to increase the potency of inactivated rabies virus vaccine, *Proc. Natl. Acad. Sci. USA* **86**:4240–4243.
- Ho, R. J. Y., *et al.*, 1991, A potentially useful vaccine adjuvant, in: *Topics in Vaccine Adjuvant Research* (D. R. Spriggs and W. C. Koff, eds.), CRC Press, Boca Raton, pp. 69–76.
- Ho, R. J., *et al.*, 1992, Liposome-formulated interleukin-2 as an adjuvant for the treatment of recurrent genital HSV-2 in guinea pigs with recombinant HSV glycoprotein gD, *Vaccine* **10**:209–213.
- Hughes, H. P. A., *et al.*, 1991, Immunopotentiation of bovine herpes virus subunit vaccination by IL-2, *Immunology* **74**:461–466.
- Hughes, H. P. A., *et al.*, 1992, Multiple administration of with cytokines potentiates antigen specific responses to subunit vaccination with bovine herpes virus-1 glycoprotein IV, *Vaccine* **10**:226–230.
- Tan, L., and Gregoriadis, G., 1989, Effect of interleukin-2 on the immunoadjuvant action of liposomes, *Biochem. Soc. Trans.* **17**:693–694.
- Mbwuike, I. N., *et al.*, 1990, Enhancement of the protective efficacy of the inactivated influenza A virus vaccine in aged mice by IL-2 liposomes, *Vaccine* **8**:347–352.
- Heath, A., Chapter 28, this volume.
- Dong, P., *et al.*, Chapter 27, this volume.

CONTACT(S): Huw Hughes, M6 Pharmaceuticals, Inc., New York, NY 10701, Ph: 212-308-7200 ext. 14; E-mail 74577.345@compuserve.com. Also: Professional Services, Chiron Therapeutics, A Division of Chiron Corporation, Emeryville, CA.

COMPONENT/ADJUVANT NAME: Interleukin-7

OTHER NAME(S): IL-7

STRUCTURE:

- Goodwin, R. G., *et al.*, 1989, Molecular cloning and growth factor activity on human and murine B-lineage cells, *Proc. Natl. Acad. Sci. USA* **86**:302–306.

Sequence of IL-7:

MFHVSFRYIFGLPPLILVLLPVASSDCDIEGKDGKQYESVLMVSIDQLLDSMKEI
GSNCLNNEFNFFKRHICDANKEGMFLFRAARKLRQFLKMNSTGDFDLHLLKV
SEGTTILLNCTGQVKGRKPAALGEAQPTKSLEENKSLKEQKKLNDLCFLKRLL
QEIKTCWNKILMGTKEH

SOURCE: Recombinant protein expressed in *E. coli*. Immunex Corp., Sterling Winthrop Pharmaceuticals.

USES: Primary adjuvant, liposome-formulated sustained release form. Co-emulsified with antigen and lipids.

APPEARANCE: Clear aqueous solution.

MOLECULAR WEIGHT: 25,000

RECOMMENDED STORAGE: 4°C for both IL-7 and liposome-formulated IL-7.

CHEMICAL/PHYSICAL PROPERTIES: Reasonable solubility in water (~1 mg/mL).

INCOMPATIBILITY: Avoid proteases.

SAFETY/TOXICITY: Unknown.

ADJUVANT PROPERTIES:

- Bui, T., *et al.*, 1994, Biologic response of recombinant interleukin-7 on herpes simplex virus infection in guinea pigs, *Vaccine* **12**:646–652.
- Bui, T., *et al.*, 1994, Effect of MTP-PE liposomes and IL-7 on induction of antibody and cell-mediated immune responses to a recombinant HIV envelope protein, *J. AIDS* (in press).
- Heath, A., Chapter 28, this volume.
- Dong, P., *et al.*, Chapter 27, this volume.

CONTACT(S): Dr. Rodney Ho, University of Washington, Seattle, WA. Ph: 206-685-3914; Fax: 206-543-3204.

COMPONENT/ADJUVANT NAME: Interleukin-12

OTHER NAME(S): IL-12; natural killer cell stimulatory factor (NKSF); cytotoxic lymphocyte maturation factor (CLMF)

STRUCTURE: IL-12 is a heterodimeric protein composed of two disulfide-bonded glycoprotein subunits approximately 35 and 40 kDa in size. The two subunits represent two separate, unrelated gene products that have to be coexpressed to yield the secreted, bioactive, heterodimeric lymphokine.

- Gubler, U., *et al.*, 1991, Coexpression of two distinct genes is required to generate secreted, bioactive cytotoxic lymphocyte maturation factor. *Proc. Natl. Acad. Sci. USA* **88**:4143–4147.
- Wolf, S. F., *et al.*, 1991, Cloning of cDNA for natural killer cell stimulatory factor, a heterodimeric cytokine with multiple biologic effects on T and natural killer cells. *J. Immunol.* **146**:3074–3081.
- Schoenhaut, D. S., *et al.*, 1992, Cloning and expression of murine IL-12. *J. Immunol.* **148**:3433–3440.

Sequence of 40-kDa subunit of human IL-12:

IWELKKDVYVVELDWYPDAPGEMVVLTCDTPEEDGITWTLDQSSEVLGSGKT
LTIQVKEFGDAGQYTCHKGGEVLSHSLLLLHKKEDGIWSTDILKDQKEPKNKT
FLRCEAKNYSGRFTCWWLTTISTDLTFSVKSSRGSSDPQGVTCGAATLSAERVR
GDNKEYEYSVECQEDSACPAAEESLPIEVMVDAVHKLKYENYTSSFFIRDIIKP
DPPKNLQLKPLKNSRQVEVSWEYPDTWSTPHSYFSLTFCVQVQGKSKREKKD
RVFTDKTSATVICRKNASISVRAQDRYYSSSWSEWASVPCS

Sequence of 35-kDa subunit of human IL-12:

RNLPVATPDPGMFPCLHHSQNLLRAVSNMLQKARQTLEFYPCTSEEIDHEDITK
DKTSTVEACLPLELTKNESCLNSRETSFITNGSCLASRKTSFMMALCLSSIYEDL
KMYQVEFKTMNAKLLMDPKRQIFLDQNMLAVIDELMQALNFNSETVPQKSSL
EEPDFYKTKIKLCILLHAFRIRAVTIDRVTSYLNAS

SOURCE: Recombinant protein purified from the medium of cultures of CHO cells transfected with IL-12 cDNAs. Natural sources of the protein include activated monocyte/macrophages and B lymphocytes.

USES: Included as a primary adjuvant component to enhance Th1-dependent cell-mediated immunity.

MOLECULAR WEIGHT: Protein: 57,200; glycosylated protein: ~70,000.

RECOMMENDED STORAGE: Store IL-12 at –70°C in pH 7 buffer free of calcium, magnesium, and potassium salts (1 mg/mL). Maximum two freeze-thaws pending further investigation. Storage in opaque polypropylene containers is preferable.

CHEMICAL/PHYSICAL PROPERTIES: Three major bands in the pI range of 4.5 to 5.3. IL-12 is most stable at pH 7 at 10 μg/mL and greater. At lower and higher pH, significant loss occurs via either protein breakdown or protein adsorption to glass. Stress conditions such as heating and shaking promote aggregation and protein loss.

INCOMPATIBILITY: Avoid proteases.

SAFETY/TOXICITY: Clinical trials are in progress in AIDS and oncology (GI and Roche). In mice and primates repetitive daily dosing with ≥50 μg/kg may result in anemia, leukopenia, hepatotoxicity, skeletal muscle necrosis (seen only in mice), and vascular leak. It has also been shown in some species that repetitive daily dosing of >1 μg/kg results in the same side effects.

ADJUVANT PROPERTIES: Enhances Th1-dependent cell-mediated immune responses, including cytolytic T-lymphocyte responses. Suppresses Th2-dependent humoral

immune responses such as IgE responses but may enhance production of Ig isotypes, such as IgG2a in mice, associated with Th1 responses.

- Afonso, L. C. C., *et al.*, 1994, The adjuvant effect of interleukin-12 in a vaccine against *Leishmania major*, *Science* **263**:235–237.
- Gately, M. K., *et al.*, 1994, Administration of recombinant IL-12 to normal mice enhances cytolytic lymphocyte activity and induces production of IFN-γ *in vivo*, *Int. Immunol.* **6**:157–167.
- McKnight, A. J., *et al.*, 1994, Effects of IL-12 on helper T cell-dependent immune responses in vivo, *J. Immunol.* **152**:2172–2179.
- Heath, A., Chapter 28, this volume.
- Dong, P., *et al.*, Chapter 27, this volume.

CONTACTS: Maury K. Gately or Alvin S. Stern, Hoffmann–LaRoche Inc., Nutley, NJ 07110-1199, Ph: 201-235-5720; Fax: 201-235-5279. Also: Stan Wolf, Genetics Institute, Cambridge, MA, Ph: 617-498-8134; Fax: 617-876-1504.

COMPONENT/ADJUVANT NAME: ISCOM(s)™

OTHER NAME(S): Immune stimulating complexes

STRUCTURE: ISCOMs are a complex composed of typically 0.5% Quillaja saponins, 0.1% cholesterol, 0.1% phospholipid, and antigen in PBS. Occasionally, surfactants are used to prepare ISCOMs (such as Mega 10) but are removed from the final formulation before use.

SOURCE: The adjuvant-active components of ISCOMs are derived by aqueous extraction of the bark of *Quillaja saponaria* and are further purified by chromatography. Quil A is a purified form of this. Further chromatographic purification provides components with high adjuvant activity and ISCOM-forming properties (see Iscoprep 7.0.3™).

USES: ISCOMs are powerful immunomodulators. Steric presentation of epitopes, and CTL responses are maximized by the incorporation of immunogens into ISCOMs. Iscotec holds patents covering the use of ISCOMs (ISCOM Basic, EPC 83850273; MATRIX, EPC 89911115.7).

APPEARANCE: ISCOMs form a clear product in solution.

MOLECULAR WEIGHT: A selection of components with various molecular weights: cholesterol, 386.7; Quil A, ~2000; DMPC, 677.9.

RECOMMENDED STORAGE: Store ISCOMs at conditions compatible with the incorporated antigen(s). In general, storage in physiological buffers 4–8°C or may be stored at –70°C.

CHEMICAL/PHYSICAL PROPERTIES: ISCOMs are stable complexes which show good suspendability (>100 mg/mL) in buffer.

INCOMPATIBILITY: Avoid exposure to alkaline pH > 8.0.

SAFETY/TOXICITY: Studies in progress have shown no adverse effects in several animal species. ISCOMs have not shown hemolytic activity at normally administered dose levels.

ADJUVANT PROPERTIES: The ISCOM is an antigen-presenting structure and has been studied for a number of antigens. ISCOMs generate long-lasting biologically functional antibody response, even in the presence of maternal antibodies. Protective immunity and a functional cell-mediated immune response, including class I-restricted CTLs, have been reported in several systems. ISCOMs have generally been administered subcutaneously or intramuscularly but nonparenteral administrations (intranasal and oral) have also proven to be effective.

- Morein, B., *et al.*, 1984, Iscom, a novel structure for antigenic presentation of membrane proteins from enveloped viruses, *Nature* **308**:457.
- Classen, I., and Osterhaus, A., 1992, The iscom structure as an immune enhancing moiety: Experiences in viral systems, *Res. Immunol.* **143**:531–541.
- Hoglund, S., *et al.*, 1989, Iscoms and immunostimulation with viral antigens, in: *Subcellular Biochemistry* (J. R. Harris, ed.), Plenum Press, New York, p. 39.
- Rimmelzwaan, G. F., and Osterhaus, A. D. M. E., Chapter 23, this volume.

CONTACT(S): ISCOTEC AB, Box 7418, S-10391 Stockholm, Sweden, Ph: 46-8-6797810; Fax: 46-18-674376. Also: Bror Morein, National Veterinary Institute, Uppsala, Sweden, Ph: 46-18-174571; Fax: 46-18-504-603.

COMPONENT/ADJUVANT NAME: Iscoprep 7.0.3™

OTHER NAME(S): None

STRUCTURE: Complex of saponin derivatives.

SOURCE: Purified by aqueous extraction of the bark of *Quillaja saponaria* and further purified by chromatography to produce Iscoprep 7.0.3™, a carefully selected mixture of saponin components with adjuvant activity and ISCOM-forming capacity.

USES: Iscoprep 7.0.3™ is used to produce ISCOMs.

APPEARANCE: Iscoprep 7.0.3™ is a light tan to white lyophilized powder.

MOLECULAR WEIGHT: Iscoprep 7.0.3™ is a selection of components with various molecular weights around 1800 to 2200.

RECOMMENDED STORAGE: Store powder at 4–8°C in the dark, solutions at –20°C.

CHEMICAL/PHYSICAL PROPERTIES: The mixture of saponin components in Iscoprep 7.0.3™ binds to cholesterol and phospholipid to form ISCOMs. ISCOMs are stable complexes made up of amphiphilic molecules.

INCOMPATIBILITY: Avoid exposure to alkaline pH >8.

SAFETY/TOXICITY: Studies are in progress. Iscoprep 7.0.3™ does not show hemolytic activity in ISCOMs at normally administered dose levels.

ADJUVANT PROPERTIES: Iscoprep 7.0.3™ is used to formulate ISCOMs. The ISCOM as an antigen-presenting structure and has been studied for a number of antigens. ISCOMs generate long-lasting biologically functional antibody response, even in the presence of maternal antibodies. Protective immunity and a functional cell-mediated immune response, including class I-restricted CTLs, have been reported in several systems. ISCOMs have generally been administered subcutaneously or intramuscularly but nonparenteral administrations (intranasal and oral) have also proven to be effective.

- Morein, B., *et al.*, 1984, Iscom, a novel structure for antigenic presentation of membrane proteins from enveloped viruses, *Nature* **308**:457.
- Classen, I., and Osterhaus, A., 1992, The iscom structure as an immune enhancing moiety: Experiences in viral systems, *Res. Immunol.* **143**:531–541.
- Hoglund, S., *et al.*, 1989, Iscoms and immunostimulation with viral antigens, in: *Subcellular Biochemistry* (J. R. Harris, ed.), Plenum Press, New York, p. 39.
- Rimmelzwaan, G. F., and Osterhaus, A. D. M. E., Chapter 23, this volume.

CONTACT(S): ISCOTEC AB, Box 7418, S-10391 Stockholm, Sweden, Ph: 46-8-6797810; Fax: 46-18-674376.

COMPONENT/ADJUVANT NAME: Liposomes

OTHER NAME(S): Liposomes (L) containing protein or Th-cell and/or B-cell peptides, or microbes with or without co-entrapped interleukin-2, BisHOP or DOTMA (see below). A, [L (Antigen)]; B, [L (IL-2 or DOTMA or BisHOP + Antigen)]; C, [L (Antigen)-mannose]; D, [L (Th-cell and B-cell epitopes)]; E, [L (microbes)].

STRUCTURE(S): A: Multilamellar liposomes prepared by the dehydration–rehydration method (average diameter 600–800 nm) composed of egg phosphatidylcholine (PC) or distearoyl phosphatidylcholine (DSPC) and equimolar cholesterol and containing antigens such as tetanus toxoid and synthetic Th-cell peptides. B: As in A with IL-2 (10^3–10^4 Cetus units) co-entrapped with the antigen in the aqueous phase or with 1,2-bis (hexadecylcycloxy)-3-trimethylaminopropane-HCL (BisHOP) or *N*-(2,3-dioleyloxy)-*N*,*N*,*N*-triethylammonium (DOTMA) incorporated into the lipid phase of liposomes (0.8:1.0:0.2 molar ratio for PC or DSPC, cholesterol and DOTMA or BisHOP). C, as in A with mannosylated albumin covalently coupled to the surface of antigen-containing liposomes. D: As in A with Th-cell and B-cell peptides co-entrapped in the aqueous phase. E: Giant liposomes (average diameter 5–9 μm) prepared as in A or by a solvent-spherule evaporation method, composed of PC or DSPC, cholesterol, triolein (TO), and phosphatidylglycerol (PG) (4:4:1:2 molar ratio) and containing killed or live *Bacillus subtilis* or killed bacille Calmette-Guérin (BCG) with or without co-entrapped tetanus toxoid.

SOURCE: PC, DSPC, and PG in pure form from Lipid Products, Nuthill, Surrey, U.K.; TO in pure form from Sigma Chemical Co., Poole, Dorset, U.K.; recombinant interleukin-2 (des-Ala$_1$-Ser$_{125}$ mutein; 3×10^6 Cetus units/mg) obtained from Cetus Corporation, Emeryville, CA; BisHOP and DOTMA obtained from Syntex Research, Palo Alto, CA.

APPEARANCE: White, opalescent colloidal suspensions (A–E).

MOLECULAR WEIGHT: Equal to the sum of the molecular weights of the components used in each of the formulations. The molecular weight of antigen will vary according to its type.

RECOMMENDED STORAGE: Store formulations at 4°C when in liquid form. Freeze-dried formulations stored at 4 or –20°C. Liquid formulations stable (in terms of entrapped antigen release) for at least 1 year when sterile. Precipitated liposomes made into suspended by light vortexing.

CHEMICAL/PHYSICAL PROPERTIES: Liposomes are stable at a pH range of 1–10; however, neutral pH is recommended when cytokines and certain antigens are present. Lipid components of liposomes are soluble in chloroform and are stable for at least 1 year at –20°C.

INCOMPATIBILITY: Formulations unstable in the presence of detergents (e.g., Triton X-100).

SAFETY/TOXICITY: Liposomes as such composed of PC and cholesterol have been administered to humans in numerous clinical trials with no adverse effects. None of the formulations (A–E) have been tested in humans.

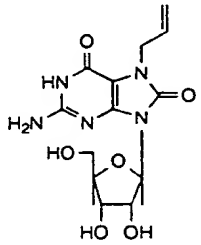
IMMUNOLOGICAL ADJUVANT AND VACCINE CARRIER PROPERTIES: A, potentiation of immune responses (IgG1, IgG2a, IgG2b, or IgG3) to protein and peptide antigens; choice of phospholipid depends on antigen; a high mass ratio of phospholipid to antigen (e.g., 10^3) optimizes immune responses. B, IL-2, DOTMA, and BisHOP potentiate immune responses to antigens further, acting as co-adjuvants. C, targets liposomes to macrophages with immune responses being greater than with conventional liposomes. D,

liposomes act as carrier of Th-cell peptide antigen which provides help for co-entrapped B-cell antigen to overcome genetic restriction and induce immunological memory. E, liposomes may act as carriers of attenuated or live microbial vaccines to deliver microbes and co-entrapped soluble antigens or cytokines simultaneously to antigen-presenting cells or to protect entrapped vaccines from interaction with maternal antibodies or antibodies to vaccine impurities in preimmunized subjects.

- Gregoriadis, G., 1990, Immunological adjuvants: A role for liposomes, *Immunol. Today* **11**:89–97.
- Davis, D., and Gregoriadis, G., 1987, Liposomes as adjuvants with immunopurified tetanus toxoid: Influence of liposomal characteristics, *Immunology* **61**:229–234.
- Gregoriadis, G., *et al.*, 1987, Liposomes as immunological adjuvants: Antigen incorporation studies. *Vaccine* **5**:143–149.
- Tan, L., and Gregoriadis, G., 1989, The effect of interleukin-2 on the immunoadjuvant action of liposomes, *Biochem. Soc. Trans.* **17**:693–694.
- Garcon, N., *et al.*, 1988, Targeted immunoadjuvant action of tetanus toxoid-containing liposomes coated with mannosylated albumin, *Immunology* **64**:743–745.
- Kahl, K. L., *et al.*, 1989, Vaccination against murine cutaneous leishmaniasis using L. Major antigen/liposomes: Optimization and assessment of the requirement for intravenous immunization, *J. Immunol.* **142**:4441–4449.
- Antimisiaris, S., *et al.*, 1993, Liposomes as vaccine carriers: Incorporation of soluble and particulate antigens in giant vesicles, *J. Immunol. Methods* **166**:271–280.
- Gregoriadis, G., *et al.*, 1993, Liposome-entrapped T-cell peptide provides help for co-entrapped B-cell peptide to overcome genetic restriction in mice and induce immunological memory, *Immunology* **80**:535–540.

CONTACT(S): Professor Gregory Gregoriadis. Centre for Drug Delivery Research, School of Pharmacy, University of London, 29-39 Brunswick Square, London WC1N 1AX, U.K., Ph:, +44-171-7535822; Phone/Fax +44-171-7535820.

COMPONENT/ADJUVANT NAME: Loxoribine
OTHER NAME(S): 7-Allyl-8-oxoguanosine
STRUCTURE:



SOURCE: Synthetic (U.S. Patent 5,011,828).

USES: Primary adjuvant for antibody responses to a wide variety of antigen types in a variety of species. Typical dose in mice: 1–3 mg/25 g mouse. In humans: 10 mg/kg has been used safely. Optimal dose unknown.

APPEARANCE: White, odorless crystalline powder.

MOLECULAR WEIGHT: 339

RECOMMENDED STORAGE: Store solid under low humidity at –20°C. Stable in solution for at least 4 weeks; found to be very stable at pH 8–11.

CHEMICAL/PHYSICAL PROPERTIES: Hydrophobic, lipophilic molecule. Soluble in DMSO, DMF, and aqueous media at alkaline pH. Mp = 234°C, pKa = 8.92.

INCOMPATIBILITY: Precipitates at low pH.

SAFETY/TOXICITY: Phase I clinical trial complete, without toxicity greater than grade I. No reported toxicity in a lower dose, Phase I/II clinical trial. Main side effects noted resemble those of interferon and are transient.

ADJUVANT PROPERTIES: Augmentation of CTL-mediated, NK cell-mediated, macrophage-mediated, and LAK cell-mediated cytotoxicity. Inducer of cytokines: IFN$\alpha/\beta/\gamma$, TNFα, TNFβ, IL-1α, IL-6. Upregulates humoral immune responses under conditions of normal immunity as well as in immunodeficiency. Acts as a surrogate Th signal.

- Goodman, M. G., and Weigle, W. O., 1985, Enhancement of the human antibody response by C8-substituted guanine ribonucleosides in synergy with interleukin 2, *J. Immunol.* **135**:3284–3288.
- Feldbush, T. L., and Ballas, Z. K., 1985, Lymphokine-like activity of 8-mercaptoguanosine: Induction of T and B cell differentiation, *J. Immunol.* **134**:3204–3211.
- Goodman, M. G., *et al.*, 1991, C-kinase independent restoration of specific immune responsiveness in common variable immunodeficiency, *Clin. Immunol. Immunopathol.* **59**:26–36.
- Goodman, M. G., and Weigle, W. O., 1983, T cell-replacing activity of C8-derivatized guanine ribonucleosides, *J. Immunol.* **130**:2042–2045.
- Pope, B. L., *et al.*, 1993, Loxoribine (7-allyl-8-oxoguanosine) activates natural killer cells and primes cytolytic precursor cells for activation by IL-2, *J. Immunol.* **151**:3007–3017.
- Goodman, M., Chapter 25, this volume.

CONTACT(S): Dr. Michael G. Goodman, Department of Immunology, The Scripps Research Institute, La Jolla, CA 92037, Ph: 619-554-8131; Fax: 619-554-6705.

COMPONENT/ADJUVANT NAME: LT-OA or LT Oral Adjuvant

OTHER NAME(S): *E. coli* labile enterotoxin protoxin

STRUCTURE: Polypeptide consisting of one 28-kDa A subunit (toxic component consisting of the A_1 chain of 21 kDa and the A_2 chain of 7 kDa) and five 11.6-kDa B subunits (binding component).

- Sixma, T. K., *et al.*, 1991, Crystal structure of a cholera toxin-related heat-labile enterotoxin from *E. coli*, *Nature* **351**:371–377.

Sequence of *E. coli* heat-labile enterotoxin subunit A:

NGDKLYRADSRPPDEIKRSGGLMPRGHNEYFDRGTQMNINLYDHARGTQTGF
VRYDDGYVSTSLSLRSAHLAGQSILSGYSTYYIYVIATAPNMFNVNDVLGVYS
PHPYEQEVSALGGIPYSQIYGWYRVNFGVIDERLHRNREYRDRYYRNLNIAPA
EDGYRLAGFPPDHQAWREEPWIHHAPQGCGDSSRTITGDTCNEETQNLSTIYL
RKYQSKVKRQIFSDYQSEVDIYNRIRNEL*

Sequence of *E. coli* heat-labile enterotoxin subunit B:

APQSITELCSEYRNTQIYTINDKILSYTESMAGKREMVIITFKSGATFQVEVPGS
QHIDSQKKAIERMKDTLRITYLTETKIDKLCVWNNKTPNSIAAISMEN*

SOURCE: Toxigenic *Escherichia coli*, either partially purified or recombinant, extracted under conditions that inhibit proteolysis and thus inhibit conversion to active toxin. Commercially available from Berna Products Corp., Coral Gables, FL.

USES: Sole active component of orally administered adjuvant.

APPEARANCE: White, odorless powder.

MOLECULAR WEIGHT: 84,000 to 94,000, depending on the assay method.

RECOMMENDED STORAGE: Store lyophilized solid at –20°C. Solutions of LT-OA at 1 mg/mL at pH 7.5 in nonphosphate buffers may be stored at 5°C for 1–2 years.

CHEMICAL/PHYSICAL PROPERTIES: Amphiphilic molecule with low solubility in water at neutral pH. pI 8.0. Not stable in phosphate buffers. Oxidizes on long-term storage to form intramolecular disulfide bonds.

INCOMPATIBILITY: Avoid proteases. Incompatible with phosphate buffer.

SAFETY/TOXICITY: Nontoxic at adjuvant-active doses in mouse, rabbit, and monkey. Human Phase I clinical trials began September, 1994.

- Majde, J. A., *et al.*, 1994, *Escherichia coli* heat-labile enterotoxin, an oral adjuvant for protection against mucosal pathogens, in: *Adjuvants—Theory and Practical Applications* (D. Stewart-Tull, ed.), Wiley, New York, pp. 337–351.

ADJUVANT PROPERTIES: For inducing mucosal and systemic immunity [both humoral (including IgA and IgG2a isotypes) and cell-mediated] to killed microorganism or peptide antigens mixed with it in neutral non-phosphate-buffered saline, with/without sodium bicarbonate.

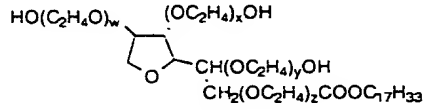
- Clements, J. D., *et al.*, 1988, Adjuvant activity of *Escherichia coli* heat-labile enterotoxin and effect on the induction of oral tolerance in mice to unrelated protein antigens, *Vaccine* **6**:269–277.
- Walker, R. I., and Clements, J. D., 1993, Use of the heat labile toxin of enterotoxigenic *Escherichia coli* to facilitate mucosal immunization, *Vaccine Res.* **2**:1–10.
- Rollwagen, F. M., *et al.*, 1993, Killed *Campylobacter* elicits immune response and protection when administered with an oral adjuvant, *Vaccine* **11**:1316–1319.
- Baqar, S., *et al.*, 1995, Safety and immunogenicity of a prototype oral whole cell killed *Campylobacter* vaccine administered with a mucosal adjuvant in non-human primates, *Vaccine* (in press).

CONTACT(S): Dr. Jeannine A. Majde, Program Manager, Biomedical Science and Technology, Office of Naval Research, Arlington, VA 22217-5660, Ph: 703-696-4055; Fax: 703-696-1212; E-mail: majdej@onrhq.onr.navy.mil.

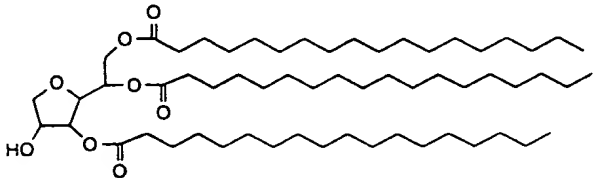
COMPONENT/ADJUVANT NAME: MF59

OTHER NAME(S): None

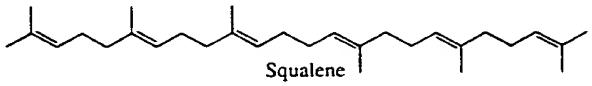
STRUCTURE: Squalene/water emulsion. Composition: 43 mg/mL squalene, 2.5 mg/mL polyoxyethylene sorbitan monooleate (Polysorbate 80), 2.4 mg/mL sorbitan trioleate (Span 85).



Polysorbate 80



Span 85



Squalene

SOURCE: The Biocine Company, Emeryville, CA.

USES: Intramuscular adjuvant.

APPEARANCE: White liquid.

MOLECULAR WEIGHT: Not applicable.

RECOMMENDED STORAGE: 2–8°C, inert gas overlay.

CHEMICAL/PHYSICAL PROPERTIES: Low-viscosity aqueous emulsion, biodegradable. Particle size 200–300 nm.

INCOMPATIBILITY: Unstable on freezing. Exposure to pH extremes results in hydrolysis of detergent components. Components are susceptible to oxidation in presence of O_2, peroxide, metals.

SAFETY/TOXICITY: Minor reactogenicity on intramuscular injection of humans in combination with HSV or HIV antigens.

ADJUVANT PROPERTIES: Intramuscular injection in combination with a variety of subunit antigens results in elevated humoral response, increased T cell proliferation, and presence of cytotoxic lymphocytes.

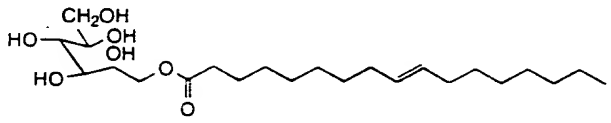
- Sanchez-Pestador, L., *et al.*, 1988, The effect of adjuvants on the efficacy of a recombinant herpes simplex glycoprotein vaccine, *J. Immunol.* **141**:1720–1727.
- Van Nest, G. A., *et al.*, 1992, Advanced adjuvant formulations for use with recombinant subunit vaccines, in: *Vaccines 92* (F. Brown, R. M. Chanock, H. S. Ginsberg, and R. A. Lerner, eds.), Cold Spring Harbor Press, Cold Spring Harbor, NY, p. 57.
- Ott, G., *et al.*, Chapter 10, this volume.

CONTACT(S): Dr. Gary Van Nest, Chiron Corporation, Emeryville, CA, Ph: 510-601-2965; Dr. Gary Ott, Chiron Corporation, Ph: 510-601-2964; Fax: 510-601-2586.

COMPONENT/ADJUVANT NAME: MONTANIDE ® ISA 51

OTHER NAME(S): Purified IFA; incomplete Freund's adjuvant

STRUCTURE: Mannide oleate (mostly mannide monooleate, esters of mannitol and oleic acids—an example is shown below) (MONTANIDE 80) in mineral oil solution (DRAKEOL 6VR).



SOURCE: Manufactured by SEPPIC.

USES: "Ready to use" oil for water-in-oil emulsion adjuvants. For human use. Final injection product usually 50% MONTANIDE ISA 51 (0.5–1 mL injection volume).

APPEARANCE: Limpid clear yellow liquid.

RECOMMENDED STORAGE: Store at 4°C or room temperature under nitrogen. Stable at room temperature for at least 1 year. Store at physiological pH.

CHEMICAL/PHYSICAL PROPERTIES: Acid value: 0.5 maximum; saponification value: between 16 and 20; hydroxyl value: between 9 and 13; peroxide value: 2 maximum; iodine value: between 5 and 9; water content: 0.5 maximum; refractive index at 25°C: between 1.455 and 1.465; density (at 20°C): about 0.85; viscosity (at 20°C): about 50 mPas. Water insoluble. DRAKEOL 6VR is a special pharmaceutical-grade mineral oil that contains paraffin oil with linear and ramified hydrocarbons in the range C_{14}–C_{26} (mean C_{24}).

INCOMPATIBILITY: Avoid strong bases.

SAFETY/TOXICITY: LD_{50} (mice) by i.p. route, > 22 g/kg. LD_{50} (rats) by oral route, > 2 g/kg. Nonirritating (skin) in rabbits, slight irritancy (ocular) in rabbits. No abnormal toxicity in mice or guinea pigs. Acute toxicity by i.m. injection (rats), > 5 g/kg. Pyrogen-free. Ames and mouse micronucleus test (Montanide 80)—no effect.

ADJUVANT PROPERTIES: Addition of MONTANIDE ISA 51 induces humoral and cell-mediated immunity with various antigens.

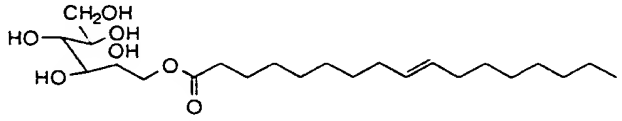
- Audibert, F. M., and Lise, L. D., 1993, Adjuvant: Current status, clinical perspectives and future prospects, *Immunol. Today* **14**:281–284.
- Ganne, V., *et al.*, 1994, Enhancement of the efficacy of a replication-defective adenovirus vectored pseudorabies vaccine by the addition of oil adjuvants, *Vaccine* (in press).

CONTACT(S): SEPPIC, 75321 Paris Cedex 07, France, Ph: 331 40 62 57 30; Fax: 331 40 62 52 53. See also monograph on Freund's incomplete adjuvant.

COMPONENT/ADJUVANT NAME: MONTANIDE ® ISA 720

OTHER NAME(S): Metabolizable oil adjuvant

STRUCTURE: A highly refined emulsifier from the mannide monooleate family (an example of mannide monooleate is shown below) in a natural metabolizable oil solution. The exact nature of the emulsifier and the metabolizable in MONTANIDE ISA 720 is proprietary, but can be disclosed under specific agreement with SEPPIC.



SOURCE: Manufactured by SEPPIC.

USES: "Ready to use" oil for water-in-oil emulsion adjuvants. Final injection product usually 70% Montanide ISA 720 (0.5–1 mL injection volume).

APPEARANCE: Yellow, odorless liquid.

RECOMMENDED STORAGE: Store at 4°C or room temperature under nitrogen. Stable at room temperature for at least 1 year. Store at physiological pH.

CHEMICAL/PHYSICAL PROPERTIES: Acid value 0.5 maximum; saponification value: between 17 and 21; hydroxyl value: between 9 and 12; peroxide value: 5 maximum; iodine value: between 320 and 350; water content: 0.5 maximum; refractive index (at 25°C) about 1.492; density (at 20°C): about 0.86; viscosity (at 20°C): about 15 mPas. Water insoluble. DRAKEOL 6VR is a special pharmaceutical-grade mineral oil that contains paraffin oil with linear and ramified hydrocarbons in the range C_{14}–C_{26} (mean C_{24}).

INCOMPATIBILITY: None found.

SAFETY/TOXICITY: LD_{50} (mice) by i.p. route, > 22 g/kg. LD_{50} (rats) by oral route, > 2 g/kg. Nonirritating (skin) in rabbits, slight irritancy (ocular) in rabbits. No abnormal toxicity in mice or guinea pigs. Acute toxicity by i.m. injection (rats), > 5 g/kg. Pyrogen-free. Ames and mouse micronucleus test (Montanide 80)—no effect.

ADJUVANT PROPERTIES: Addition of MONTANIDE ISA 720 induces humoral and cell-mediated immunity with various antigens.

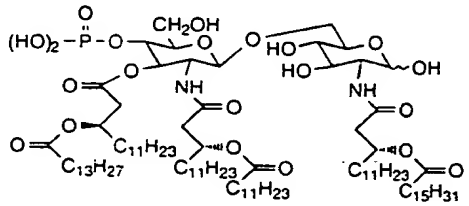
- Jones, W. R., *et al.*, 1988, Phase I clinical trial of World Health Organization birth control vaccine, *Lancet* 1:1295–1298.
- Jones, G. L., *et al.*, 1990, Peptide vaccines derived from a malarial surface antigen: Effects of dose and adjuvants on immunogenicity, *Immunol. Lett.* 24:253–260.
- Elliot, S., *et al.*, 1994, Human compatible adjuvant induces protective cytotoxic T lymphocytes with peptide vaccine, Proceedings in CHI Vaccines—New Technologies and Applications, Alexandria, VA, March 21–23.

CONTACT(S): SEPPIC, 75321 Paris Cedex 07, France, Ph: 331 40 62 57 30; Fax: 331 40 62 52 53.

COMPONENT/ADJUVANT NAME: MPL®

OTHER NAME(S): 3-*O*-desacyl-4′-monophosphoryl lipid A; 3D-MLA

STRUCTURE: MPL® is composed of a series of 4′-monophosphoryl lipid A species that vary in the extent and position of fatty acid substitution. The hexaacyl structure shown below is the most highly acylated and most abundant component in MPL®. Species with five and four fatty acids are also present. All structures contribute to the adjuvant activity of MPL®.



SOURCE: Derived from the lipopolysaccharide (LPS) of *Salmonella minnesota* R595. Obtained by treatment of LPS with mild acid and base hydrolytic conditions, and chromatographic purification of the resulting 3D-MLA.

USES: As a primary adjuvant in adjuvant formulations. Adjuvant activity is manifested either alone in aqueous solution with antigen, or in combination with particulate vehicles (e.g., oil-in-water emulsions). Activity may be enhanced by use of vehicle that enforces close association with antigen.

APPEARANCE: Colorless, odorless white powder.

MOLECULAR WEIGHT: 1540–1670 (average).

RECOMMENDED STORAGE: Indefinite stability as lyophilized powder (in excess of 5 years if stored at 5°C). Available data indicate stability in aqueous solution is maximum between pH 5 and 6. An aqueous formulation was stable (<10% loss of most highly acylated component) for the equivalent of 2–3 years in an accelerated stability study.

CHEMICAL/PHYSICAL PROPERTIES: Composed of closely related 4′-monophosphoryl lipid A species that vary only in terms of fatty acid content (see above). All species in MPL® are highly amphiphilic. MPL® is probably aggregated in aqueous solution at concentrations above ~1 nM. Micelles or liposomes are formed depending on excipients and conditions of formulation. Solubility in water is greatest above pH 7, and is diminished below pH 5. Surfactants enhance solubility in water. Soluble in oils (e.g., squalene).

INCOMPATIBILITY: Solubility is diminished significantly in the presence of divalent metal cations.

SAFETY/TOXICITY: Has been studied in human Phase I/II clinical trials. Results to date indicate that MPL® is well tolerated at doses that exhibit beneficial immunostimulating activities. MPL® is pyrogenic at high doses.

- Rudbach, J. A., *et al.*, 1994, Prophylactic use of monophosphoryl lipid A in patients at risk for sepsis, in: *Bacterial Endotoxins: Basic Science to Anti-Sepsis Strategies-Proceedings of the International Conference on Endotoxins IV* (J. Levin, A. Sturk, T. Van Der Poll, and S. J. H. Van Deventer, eds.), Wiley, New York (in press).
- Thoelen, S., *et al.*, 1993, Immunogenicity of a recombinant hepatitis B vaccine with monophosphoryl lipid A administered following various two-dose schedules, Abstr. 340, 33rd Intersci. Conf. Antimicrob. Agents Chemother., p. 182.
- Van Damme, P., *et al.*, 1993, Safety, humoral and cellular immunity of a recombinant hepatitis B vaccine with monophosphoryl lipid A in healthy volunteers, Abstr. 667, 33rd Intersci. Conf. Antimicrob. Agents Chemother., p. 241.

ADJUVANT PROPERTIES: Numerous references have documented the adjuvant activity of MPL®.

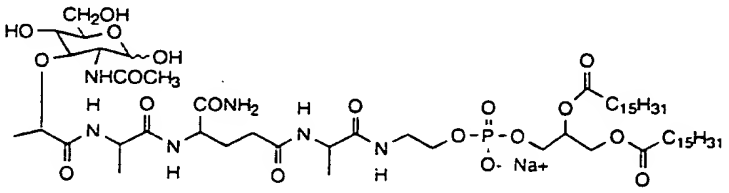
- Ulrich, J. T., *et al.*, 1991, The adjuvant activity of monophosphoryl lipid A, in: *Topics in Vaccine Adjuvant Research* (D. R. Spriggs, and W. C. Koff, eds.), CRC Press, Boca Raton, pp. 133–143.
- Ivins, B. E., *et al.*, 1992, Immunization against anthrax with *Bacillus anthracis* protective antigen combined with adjuvants, *Infect. Immun.* **60**:662–668.
- Gustafson, G. L., and Rhodes, M. J., 1992, Bacterial cell wall products as adjuvants: Early interferon gamma as a marker for adjuvants that enhance protective immunity, *Res. Immunol.* **143**:483–488.
- Baker, P. J., 1990, Regulation of magnitude of antibody response to bacterial polysaccharide antigens by thymus-derived lymphocytes, *Infect. Immun.* **58**:3465–3468.
- Ulrich, J. T., and Myers, K. R., Chapter 21, this volume.

CONTACT(S): J. T. Ulrich/K. R. Myers, Ribi ImmunoChem Research, Inc., Hamilton, MT 59840, Ph: 406-363-6214; Fax: 406-363-6129; E-mail: 74043.1020@compuserve.com.

COMPONENT/ADJUVANT NAME: MTP-PE

OTHER NAME(S): *N*-acetyl-L-alanyl-D-isoglutaminyl-L-alanine-2-(1,2-dipalmitoyl-sn-glycero-3-(hydroxy-phosphoryloxy)) ethylamide, mono sodium salt.

STRUCTURE: $C_{59}H_{108}N_6O_{19}PNa \cdot 3\ H_2O$



SOURCE: Chemical synthesis by Ciba Geigy Ltd., Basel, Switzerland.

USES: Immunomodulator. Optionally a part of MF59.

APPEARANCE: White powder.

MOLECULAR WEIGHT: 1313.55

RECOMMENDED STORAGE: 2–8°C.

CHEMICAL/PHYSICAL PROPERTIES: Amphiphilic properties. Solubility in water 1%. Biodegradable.

INCOMPATIBILITY: Hydrolysis at high or low pH values.

SAFETY/TOXICITY: MTP-PE has been injected intravenously in a liposomal formulation in cancer patients and was safe up to 6 mg/m^2. Addition of MTP-PE to the MF59 adjuvant results in increased rates of local and systemic reactions over those seen in the absence of the muramyl peptide. No evidence of uveitis was seen in any patients receiving MF59 with MTP-PE.

- Wintsh, J., *et al.*, 1991, Safety and immunogenicity of a genetically engineered human immunodeficiency virus vaccine, *J. Infect. Dis.* **163**:219.

ADJUVANT PROPERTIES: In seronegative populations, humoral and cellular responses to HSV and HIV vaccine were not enhanced when MTP-PE was included in MF59. The addition of MTP-PE to the MF59-based HIV vaccine in HIV-seropositive individuals resulted in a marked increase in HIV antigen lymphocyte proliferation.

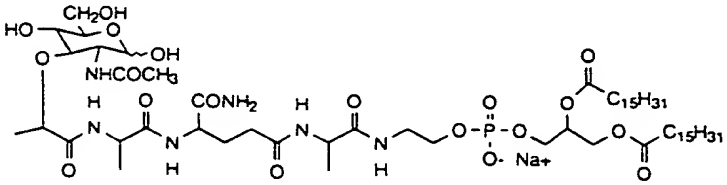
- Sanchez-Pestador, L., *et al.*, 1988, The effect of adjuvants on the efficacy of a recombinant herpes simplex glycoprotein vaccine, *J. Immunol.* **141**:1720–1727.
- Van Nest, G. A., *et al.*, 1992, Advanced adjuvant formulations for use with recombinant subunit vaccines, in: *Vaccines 92* (F. Brown, R. M. Chanock, H. S. Ginsberg, and R. A. Lerner, eds.), Cold Spring Harbor Press, Cold Spring Harbor, NY, p. 57.
- Ott, G., *et al.*, Chapter 10, this volume.

CONTACT(S): Dr. Peter van Hoogevest, Ciba Geigy Ltd., CH-4002 Basel, Switzerland. Ph: 41-61-6965651; Fax: 41-61-696-6981. Also: Gary Van Nest, Chiron Corp., Emeryville, CA. Ph: 510-601-2965; Fax: 510-601-2586.

COMPONENT/ADJUVANT NAME: MTP-PE Liposomes

OTHER NAME(S): MTP-PE antigen-presenting liposomes

STRUCTURE: $C_{59}H_{108}N_6O_{19}PNa \cdot 3H_2O$ (MTP)



SOURCE: Chemical synthesis.

- Phillip, N. C., *et al.*, 1985. Activation of alveolar macrophage tumoricidial activity and eradication of experimental metastases by freeze-dried liposomes containing a new lipophilic muramyl dipeptide derivative. *Cancer Res.* **45**:128–134.

USES: Primary adjuvant, liposome-formulated sustained release form, co-emulsified with antigen and lipids.

APPEARANCE: Lyophilized white powder.

MOLECULAR WEIGHT: MTP-PE, 1313.55

RECOMMENDED STORAGE: Store at 4°C for both the parent lyophilized compound and the liposome-formulated MTP-PE.

CHEMICAL/PHYSICAL PROPERTIES: Amphipathic molecule with good water solubility (1–2 mg/mL). It may form as mixed micelle suspension at high concentration. MTP-PE liposomes are multilamellar in nature, ranging from 1 to 5 μm in diameter.

INCOMPATIBILITY: Unknown.

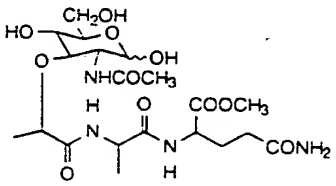
SAFETY/TOXICITY: Liposome-formulated MTP-PE is currently under Phase II/III trials. Some systemic toxicity with MTP-PE given with gp120-based HIV-1 vaccines.

ADJUVANT PROPERTIES:

- Ho, R. J. Y., *et al.*, 1989. Antigen presenting liposomes are effective in treatment of recurrent herpes simplex genitalis in guinea pigs. *J. Virol.* **63**:2951–2958.
- Ho, R. J. Y., *et al.*, 1994. Disposition of antigen-presenting liposomes in vivo: Effect on presentation of HSV antigen rgD. *Vaccine* **12**:235–242.
- Bui, T., *et al.*, 1994. Effect of MTP-PE liposomes and IL-7 on induction of antibody and cell-mediated immune responses to a recombinant HIV envelope protein. *J. AIDS* **7**:799–806.

CONTACT(S): Rodney J. Y. Ho, Department of Pharmaceutics, University of Washington, Seattle, WA 98195. Ph: 206-543-9434; Fax: 206-543-3204. Also: Dr. Peter van Hoogevest, Ciba Geigy Ltd., CH-4002 Basel, Switzerland. Ph: 41-61-6965651; Fax: 41-61-696-6981.

COMPONENT/ADJUVANT NAME: Murametide
OTHER NAME(S): NAc-Mur-L-Ala-D-Gln-OCH_3
STRUCTURE:



SOURCE: Synthesis.

USES: Administered in water-in-oil emulsion as adjuvant of humoral and cell-mediated immunity.

APPEARANCE: White powder.

MOLECULAR WEIGHT: 506.5

RECOMMENDED STORAGE: Stored as a powder at 4°C. Protect from light and humidity. Stable for more than 5 years.

CHEMICAL/PHYSICAL PROPERTIES: Hydrophilic molecule. Freely soluble in water. The solution is clear, colorless, and odorless, store at pH 5 to 7.5.

INCOMPATIBILITY: Avoid strong bases.

SAFETY/TOXICITY: Acute, subacute, and chronic toxicity studies in rats and monkeys allow administration in clinical studies of 1 to 4 s.c. injections at 2–4 week intervals at doses of 35–100 μg/kg. Pharmacokinetics was studied in rats and dogs. A Phase I clinical trial was completed that showed no toxicity at dosages up to 150 μg/kg.

ADJUVANT PROPERTIES: When administered in saline, Murametide is nonpyrogenic, induces granulocytosis, and enhances the humoral response. Murametide displays the same profile of adjuvant activity as MDP and has been chosen for development because of its favorable therapeutic ratio. When administered in 50% water-in-oil emulsion, it mimics the activity of Freund's complete adjuvant without its side effects. U.S. Patent #4,693,998.

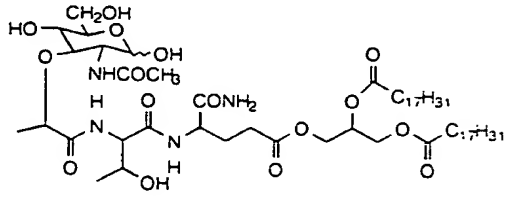
- Audibert, F., *et al.*, 1985, Muramyl peptides as immunopharmacological response modifiers, in: *Biological Response Modifiers* (P. F. Torrence, ed.), Academic Press, New York, pp. 307–337.

CONTACT(S): Professor Louis Chedid, Dr. Françoise Audibert, VACSYN S.A., 75015 Paris, France, Ph: 331-40-60-75-92; Fax: 331-40-60-75-73.

COMPONENT/ADJUVANT NAME: Murapalmitine

OTHER NAME(S): NAc-Mur-L-Thr-D-isoGln-sn-glycerol dipalmitoyl

STRUCTURE:



SOURCE: Synthesis.

USES: Administered in water-in-oil emulsion as adjuvant of humoral and cell-mediated responses.

APPEARANCE: White powder.

MOLECULAR WEIGHT: 1072

RECOMMENDED STORAGE: Stored as a powder at 4°C. Protect from light and humidity.

CHEMICAL/PHYSICAL PROPERTIES: Lipophilic molecules giving homogeneous suspensions in mineral oil.

INCOMPATIBILITY: Avoid strong bases.

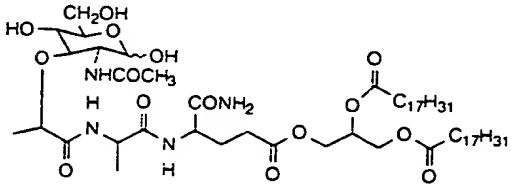
SAFETY/TOXICITY: Acute toxicity in mice, rats, rabbits, and guinea pigs satisfactorily completed. Preliminary subacute toxicity in dogs satisfactorily completed. Further toxicology studies in progress.

ADJUVANT PROPERTIES: Lipophilic MDPs are more active than hydrophilic MDPs. In saline, they are strong immunoadjuvants of humoral immunity and weaker adjuvants of cell-mediated immunity. In 50% w/o emulsions, they are strong immunoadjuvants and mimic Freund's complete activity. In contrast with other molecules of this subgroup, Murapalmitine is devoid of side effects and thus has been chosen for further development. U.S. Patents #4,939,122 and 5,210,072.

- Audibert, F., *et al.*, 1985, Muramyl peptides as immunopharmacological response modifiers, in: *Biological Response Modifiers* (P. F. Torrence, ed.), Academic Press, New York, pp. 307–337.

CONTACT(S): Professor Louis Chedid, Dr. Françoise Audibert, VACSYN S.A., 75015 Paris, France, Ph: 331-40-60-75-92; Fax: 331-40-60-75-73.

COMPONENT/ADJUVANT NAME: D-Murapalmitine
OTHER NAME(S): NAc-Mur-D-Ala-D-isoGln-sn-glycerol dipalmitoyl
STRUCTURE:



SOURCE: Synthesis.

USES: Administered in water-in-oil emulsion as adjuvant of humoral and cell-mediated immunity. To be developed as an adjuvant for vaccines likely to contain autoantigens.

APPEARANCE: White powder.

MOLECULAR WEIGHT: 1056

RECOMMENDED STORAGE: Stored as a powder at 4°C. Protect from light and humidity.

CHEMICAL/PHYSICAL PROPERTIES: Lipophilic molecules giving homogeneous suspensions in mineral oil.

INCOMPATIBILITY: Avoid strong bases.

SAFETY/TOXICITY: This MDP analogue is nonpyrogenic in rabbits and displays no acute toxicity when tested in mice, rats, guinea pigs, and rabbits. Further toxicology studies will be performed.

ADJUVANT PROPERTIES: D-Murapalmitine is a strong adjuvant of humoral and cell-mediated immunity when administered in a 50% mineral oil emulsion. However, in contrast with other MDPs, it does not induce experimental allergic encephalomyelitis (EAE) when administered with myelin basic protein. When given with an antigenic preparation containing hetero and autologous epitopes, it favors the response to the heterologous determinants. U.S. patent #4,939,122, French patent #9,207,126.

- Audibert, F., *et al.*, 1985, Muramyl peptides as immunopharmacological response modifiers, in: *Biological Response Modifiers* (P. F. Torrence, ed.), Academic Press, New York, pp. 307–337.

CONTACT(S): Professor Louis Chedid, Dr. Françoise Audibert, VACSYN S. A., 75015 Paris, France, Ph: 331-40-60-75-92; Fax: 331-40-60-75-73.

COMPONENT/ADJUVANT NAME: NAGO

OTHER NAME(S): Neuraminidase–galactose oxidase

STRUCTURE: NAGO is a mixture of the enzymes neuraminidase and galactose oxidase at a 1:5 ratio in units of activity. The primary amino acid sequences of the two enzymes are appended.

- McPherson, M. J., *et al.*, 1992, Galactose oxidase of *Dactylium dendroides*: Gene cloning and sequence analysis, *J. Biol. Chem.* **267**:8146–8152.
- Galen, J. E., *et al.*, 1992, Role of *Vibrio cholerae* neuraminidase in the function of cholera toxin, *Infect. Immun.* **60**:406–415.

Sequence of neuraminidase (E.C. 3.2.18):

MRFKNVKKTALMLAMFGMATSSNAALFDYNATGDTEFDSPAKQGWMQDNT
NNGSGVLTNADGMPAWLVQGIGGRAQWTYSLSTNQHAQASSFGWRMTTEM
KVLSGGMITNYYANGTQRVLPIISLDSSGNLVVEFEGQTGRTVLATGTAATEYH
KFELVFLPGSNPSASFYFDGKLIRDNIQPTASKQNMIVWGNGSSNTDGVAAYRD
IKFEIQGDVIFRGPDRIPSIVASSVTPGVVTAFAEKRVGGGDPGALSNTNDIITRTS
RDGGITWDTELNLTEQINVSDEFDFSDPRPIYDPSSNTVLVSYARWPTDAAQNG
DRIKPWMPNGIFYSVYDVASGNWQAPIDVTDQVKERSFQIAGWGGSELYRRN
TSLNSQQDWQSNAKIRIVDGAANQIQVADGSRKYVVTLSIDESGGLVANLNGV
SAPIILQSEHAKVHSFHDYELQYSALNHTTTLFVDGQQITTWAGEVSQENNIQF
GNADAQIDGRLHVQKIVLTQQGHNLVEFDAFYLAQQTPEVEKDLEKLGWTKI
KTGNTMSLYGNASVNPGPGHGITLTRQQNISGSQNGRLIYPAIVLDRFFLNVMS
IYSDDGGSNWQTGSTLPIPFRWKSSSILETLEPSEADMVELQNGDLLLTARLDF
NQIVNGVNYSPRQQFLSKDGGITWSLLEANNANVFSNISTGTVDASITRFEQSD
GSHFLLFTNPQGNPAGTNGRQNLGLWFSFDEGVTWKGPIQLVNGASAYSDIYQ
LDSENAIVIVETDNSNMRILRMPITLLKQKLTLSQN

Sequence of galactose oxidase (E.C. 1.1.3.9):

MKHLLTLALCFSSINAVAVTVPHKAVGTGIPEGSLQFLSLRASAPIGSAISRNNW
AVTCDSAQSGNECNKAIDGNKDTFWHTFYGANGDPKPPHTYTIDMKTTQNV
NGLSMLPRQDGNQNGWIGRHEVYLSSDGTNWGSPVASGSWFADSTTKYSNFE
TRPARYVRLVAITEANGQPWTSIAEINVFQASSYTAPQPGLGRWGPTIDLPIVPA
AAAIEPTSGRVLMWSSYRNDAFGGSPGGITLTSSWDPSTGIVSDRTVTVTKHD
MFCPGISMDGNGQIVVTGGNDAKKTSLYDSSSDSWIPGPDMQVARGYQSSAT
MSDGRVFTIGGSWSGGVFEKNGEVYSPSSKTWTSLPNAKVNPMLTADKQGLY
RSDNHAWLFGWKKGSVFQAGPSTAMNWYYTSGSGDVKSAGKRQSNRGVAP
DAMCGNAVMYDAVKGKILTFGGSPDYQDSDATTNAHIITLGEPGTSPNTVFAS
NGLYFARTFHTSVVLPDGSTFITGGQRRGIPFEDSTPVFTPEIYVPEQDTFYKQN
PNSIVRVYHSISLLLPDGRVFNGGGGLCGDCTTNHFDAQIFTPNYLYNSNGNLA
TRPKITRTSTQSVKVGGRITISTDSSISKASLIRYGTATHTVNTDQRRIPLTLTNNG
GNSYSFQVPSDSGVALPGYWMLFVMNSAGVPSVASTIRVTQ

SOURCE: GO is biochemically purified from *Dactylium dendroides*. It has an activity of approximately 800 units per mg of protein and is commercially available from Sigma Chemical Co. (Cat. No. G3385) as a partially purified lyophilized powder containing 25% protein to be reconstituted in buffered saline. NA is biochemically purified from *Vibrio cholerae*. It has an activity of 25 units per μg of protein and is commercially available from Merck Ltd. (BDH Laboratory Supplies), Merck House, Poole, Dorset BH12 1BR, U.K.

USES: Primary adjuvant. A mixture of the two enzymes containing 10 units NA and 50 units GO per mL of aqueous solution and antigen is administered subcutaneously or

intramuscularly. It is comparable in effectiveness to Freund's complete adjuvant, but is nonreactogenic. It is especially effective in the induction of CD8 cytotoxic T-cell responses.

APPEARANCE: GO: lyophilized off-white solid. NA: clear, colorless aqueous solution.

MOLECULAR WEIGHT: GO: 68,500. NA: 83,000.

RECOMMENDED STORAGE: Long-term storage: GO at –20°C, NA at 4°C.

CHEMICAL/PHYSICAL PROPERTIES: Biologically active proteins in aqueous media.

INCOMPATIBILITY: Avoid denaturing conditions. Not compatible with alum. Activity reduced in oil emulsions.

SAFETY/TOXICITY: Histopathology studies in mice showed that NAGO is less inflammatory than alum and produced no adverse local or systemic reactions.

ADJUVANT PROPERTIES: NAGO generates cell surface Schiff base-forming aldehydes on antigen-presenting cells and Th cells, thereby amplifying physiologic Schiff base formation that occurs between cell-surface ligands as an essential element in APC:T-cell inductive interaction. It is a potent noninflammatory adjuvant with viral, bacterial, and protozoal subunit vaccines, and is especially effective in the generation of cytotoxic T cells.

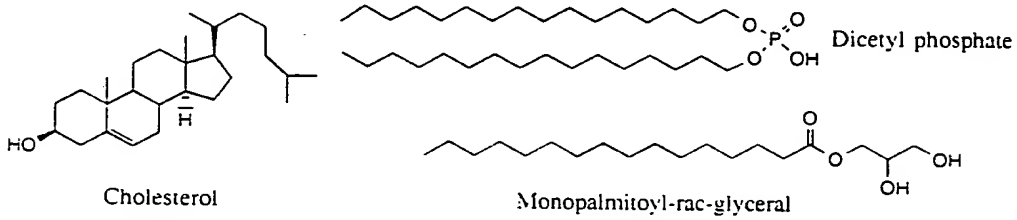
- Zheng, B., *et al.*, 1992, Galactose oxidation in the design of immunogenic vaccines, *Science* **256**:1560–1563.
- Zhong, G., *et al.*, 1993, Immunogenicity evaluation of a lipidic amino acid based synthetic peptide vaccine for *Chlamydia trachomatis*, *J. Immunol.* **151**:3728–3736.

CONTACT(S): Dr. John Rhodes, Wellcome Foundation Ltd., Beckenham, Kent BR3 3BS, U.K., Ph: 44-81-639-5336; Fax: 44-81-663-6176.

COMPONENT/ADJUVANT NAME: Nonionic Surfactant Vesicles

OTHER NAME(S): NISV

STRUCTURE: Multilamellar vesicles comprising a mixture of nonionic surfactant (e.g., 1-monopalmitoyl-rac-glycerol), cholesterol, and dicetyl phosphate.



SOURCE: Synthetic/semisynthetic.

- Alexander, J., and Brewer, J. M., 1992. Vaccines containing non-ionic surfactant vesicles, PCT/GB93/00716, priority date 7 April 92.

USES: Used as a primary vaccine adjuvant for entrapped antigen. NISV adjuvant biodegrades in vivo with release of the entrapped antigen. NISV adjuvant induces humoral and cell-mediated immunity and probably functions by targeting the antigen to the macrophage population.

APPEARANCE: Milky, colloidal suspension.

MOLECULAR WEIGHT: 1-Monopalmitoyl glycerol: 329; cholesterol: 386; dicetyl phosphate: 547.

RECOMMENDED STORAGE: Component raw materials should be stored at low humidity. Refrigeration of NISV at 4°C is preferred for antigen-containing preparations. Optimal storage conditions are under evaluation.

CHEMICAL/PHYSICAL PROPERTIES: Stable at neutral and alkaline pH. Components are amphiphilic, insoluble in water, and soluble in chloroform. The T_c of NISV is approximately 55°C. NISV are formulated as a suspension in saline.

INCOMPATIBILITY: Most organic solvents and some detergents; osmotically sensitive.

SAFETY/TOXICITY: Extremely low toxicity of NISV has been demonstrated in rat studies after administration by either the subcutaneous or intramuscular route. At doses up to 575 mg/kg body weight there was no persistence of NISV at the site of injection (s.c.).

- Brewer, J. M., *et al.*, 1994, Non-ionic surfactant vesicles as vaccine delivery systems, in: *Proceedings of the Second Conference on Industrial Immunology*, Chameleon Press, London, pp. 34–36.

ADJUVANT PROPERTIES: Induces both a humoral and cell-mediated immune response. Preferentially stimulates the Th1 subpopulation of T-helper cells. Effective with antigens within a broad size range, from short peptides to particulates. Adjuvant function is unrestricted by genetic background.

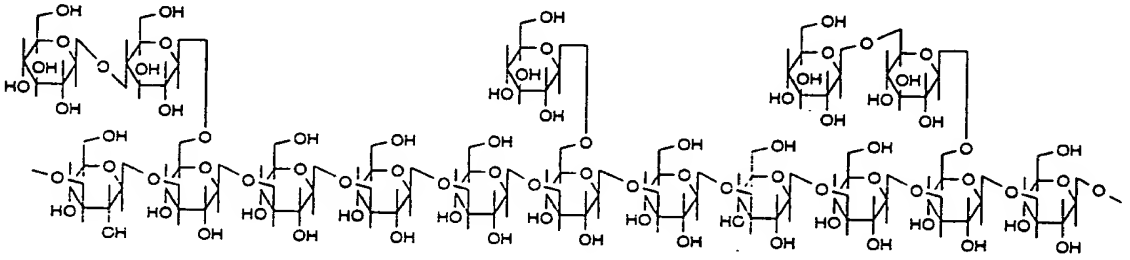
- Brewer, J. M., and Alexander, J., 1992, The adjuvant activity of non-ionic surfactant vesicles (niosomes) on the BALB/c humoral response to bovine serum albumin, *Immunology* **75**:570–575.
- Brewer, J. M., and Alexander, J., 1994, Studies on the adjuvant activity of non-ionic surfactant vesicles: Adjuvant-driven IgG2a production independent of MHC control, *Vaccine* **12**:613–619.
- Brewer, J. M., *et al.*, 1994, The demonstration of an essential role for macrophages in the in vivo generation of IgG2a antibodies, *Clin. Exp. Immunol.* **97**:164–171.

CONTACT(S): Jurek S. Sikorski, Proteus Molecular Design Limited, Macclesfield, Cheshire SK11 0JL, U.K., Ph: 44-625-500555; Fax: 44-625-500666.

COMPONENT/ADJUVANT NAME: Pleuran

OTHER NAME(S): β-glucan; glucan

STRUCTURE: A β-1,3-linked glucose polymer having β-D-glucosyl side chains attached by alternate $\beta(1,6)$ or $\beta(1,4)$ bonds at the -0-6 position of every fourth anhydroglucose unit.



SOURCE: Isolated from the fruit-body of the oyster fungus *Pleurotus ostreatus* by alkali extraction at 95–100°C, followed by bleaching with sodium chlorite (pH 3.5–4.5) at 50–60°C. The bleached products were washed in water, dehydrated in organic solvent, and finally dried by vacuum at 60°C.

- Kuniake, L., *et al.*, 1993, A new fungal glucan and its preparation, W. I. P. O. Patent No. WO93/12243.
- Karacsonyi, S., and Kuniak, L., 1994, Polysaccharides of *Pleurotus ostreatus*: Isolation and structure of Pleuran, an alkali-insoluble β-D-glucan, *J. Biopolym.* (in press).

USES: Administered with antigen for enhancement of both humoral and cell-mediated immunity. β-glucans exert their immunostimulatory activities by binding to specific β-glucan receptors on macrophages. This ligand–receptor interaction results in macrophage activation and, in certain formulations, promotes antigen targeting.

- DiLuzio, N. R., *et al.*, 1979, Evaluation of the mechanism of glucan-induced stimulation of the reticuloendothelial system, *J. Reticuloendothel. Soc.* **7**:731–742.
- Czop, J. K., and Austen, K. F., 1985, A β-glucan inhibitable receptor on human monocytes: Its identity with the phagocytic receptor for particulate activators of the alternative complement pathway, *J. Immunol.* **134**:2588–2593.

APPEARANCE: White, odorless powder. Viscous in aqueous solution.

MOLECULAR WEIGHT: 762,000

RECOMMENDED STORAGE: Stable to light. Store solid Pleuran at room temperature and aqueous suspensions at 4°C. Optimal storage conditions are to be determined.

CHEMICAL/PHYSICAL PROPERTIES: Water insoluble. Median particle size of homopolymer is 150 μm. Purified preparations contain <0.57% chitin and <0.03% protein.

INCOMPATIBILITY: Alkaline pH disrupts the triple-helical conformation.

SAFETY/TOXICITY: In preclinical studies, Pleuran has been intravenously administered at doses up to 25 mg/kg body weight and was well tolerated. Human clinical trials of β-glucans isolated from either plants or microorganisms indicate the feasibility of administering these compounds to humans without toxicity. Glucan particles bioerode over time in a physiological environment.

- Mansel, P. W. A., *et al.*, 1975, Macrophage-mediated destruction of human malignant cells in vivo, *J. Natl. Cancer Inst.* **54**:571–580.
- Okamura, K., *et al.*, 1986, Clinical evaluation of Schizophyllan combined with irradiation in patients with cervical cancer, *Cancer* **58**:865–872.
- Chihara, G., *et al.*, 1989, Lentinan as a host defense potentiator (HDP), *Int. J. Immunother.* **4**:145–154.

• Ostroff, G. R., 1994, Future therapeutic applications of Betafectin, a carbohydrate-based immunomodulator, The Second Annual Conference on Glycotechnology.

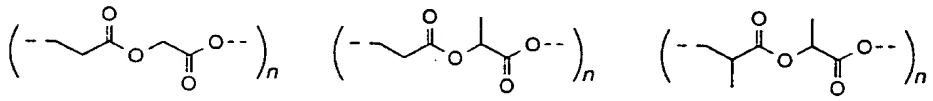
ADJUVANT PROPERTIES: Rabbits as well as mice immunized once by coadministration of viral antigens and 60 μg of Pleuran produced at least 20-fold higher antibody titers than control animals injected with the immunogen alone.

CONTACT(S): Richard McIntosh, Genesis Technology Group, Inc., Cambridge, MA 02139, Ph: 617-576-6610; Fax: 617-876-4002. Also: Dr. Nahid Mohagheghpour, SRI International, Menlo Park, CA 94025, Ph: 415-859-3516; Fax: 415-859-3342.

COMPONENT/ADJUVANT NAME: PLGA, PGA, and PLA

OTHER NAME(S): Homo- and copolymers of lactic and glycolic acid; lactide/glycolide polymers; poly-lactic-co-glycolide

STRUCTURE: Structures shown below (left to right): PGA, homo-PLGA, and PLA.



SOURCE: Synthesized by the ring-opening polymerization of the cyclic dimers, lactide and glycolide.

- Deasy, P. B., *et al.*, 1989. Preparation and characterization of lactic/glycolic acid polymers and copolymers. *J. Microencapsul.* **6**:369–378.

USES: Antigens incorporated in PLGA microspheres have exhibited enhanced and prolonged immune responses compared to equivalent doses of free antigen.

APPEARANCE: Odorless, white to tan pellets.

MOLECULAR WEIGHT: Standard grades available from 10,000 to 500,000.

RECOMMENDED STORAGE: Store at 0°C or below and minimize exposure to moisture to maintain quality.

CHEMICAL/PHYSICAL PROPERTIES: Stable except in presence of moisture. Polymers react with water and degrade to glycolic and/or lactic acid.

INCOMPATIBILITY: Reacts with water and aqueous acids and bases. Hydrolyzes to form hydroxyacetic acid (glycolic acid) and lactic acid.

SAFETY/TOXICITY: Materials are used commercially as surgical suture, staples and clips, and sustained release delivery systems. FDA has approved specific applications using this family of polymers. Drug Master File (DMF) established with FDA.

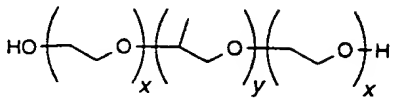
ADJUVANT PROPERTIES: The adjuvant properties of polylactides have been ascribed to the size of the microspheres and small microspheres (<10 μm) may be phagocytosed to enhance antigen presentation. However, the major use of polylactides for vaccine delivery is based on their ability to control the release of antigen after administration, thereby eliminating or reducing the need for boost immunizations.

- Cleland, J. L., *et al.*, 1994. Development of a single shot subunit vaccine for HIV-1. *AIDS Res. Hum. Retroviruses* **10**:S21–S26.
- Eldridge, J. H., *et al.*, 1991. Biodegradable and biocompatible poly(DL-lactide-co-glycolide) microspheres as an adjuvant for staphylococcal enterotoxin B toxoid which enhances the level of toxin-neutralizing antibodies. *Infect. Immun.* **59**:2978–2986.
- Eldridge, J. H., *et al.*, 1991. Biodegradable microspheres as a vaccine delivery, *Mol. Immunol.* **28**:287–294.
- Singh, M., *et al.*, 1992. Immunogenicity studies on diphtheria toxoid loaded biodegradable microspheres, *Int. J. Pharm.* **85**:R5–R8.
- Hazrati, A. M., *et al.*, 1993. Studies of controlled delivery tetanus vaccine in mice, *Proc. Int. Symp. Control. Rel. Bioact. Mater.* **20**:67–68.
- Hazrati, A. M., *et al.*, 1993. *Salmonella enteritidis* vaccine utilizing biodegradable microspheres, *Proc. Int. Symp. Control. Rel. Bioact. Mater.* **20**:101–102.
- Aguado, M. T., and Lambert, P. -H., 1992. Controlled-release vaccines—biodegradable polylactide/polyglycolide (PL/PG) microspheres as antigen vehicles, *Immunobiology* **184**:113–125.
- Esparza, I., and Kissel, T., 1992. Parameters affecting the immunogenicity of microencapsulated tetanus toxoid. *Vaccine* **10**:176–180.

- Nellore, R. V., *et al.*, 1992, Evaluation of biodegradable microspheres as vaccine adjuvant for hepatitis B surface antigen, *J. Parenteral Sci. Tech.* **46**:176–180.
- Hanes, J., *et al.*, Chapter 16, this volume.
- Cleland, J. L., Chapter 18, this volume.

CONTACT(S): There are several suppliers of PLGA polymers. The monograph prepared by: Medisorb Technologies Intl. L. P., Cincinnati, OH 45242. Ph: 800-772-5091; Fax: 513-489-7244. Also: Jeffrey L. Cleland, Genentech, Inc., South San Francisco, CA 94080, Ph: 415-225-3921; Fax: 415-225-2866; E-mail: cleland.jeffrey@gene.com.

COMPONENT/ADJUVANT NAME: Pluronic L121
OTHER NAME(S): Poloxamer 401
STRUCTURE:



SOURCE: Synthetic block copolymer of ethylene oxide and propylene oxide.

USES: Component of IDEC Antigen Formulation (AF) present in final concentration of 0.05–1.25% (w/v) with antigen, and as a component of the Syntex Adjuvant Formulation (SAF) present in a final concentration of 2.5% (w/v) with antigen.

APPEARANCE: Off-white viscous liquid at room temperature.

MOLECULAR WEIGHT: Approximately 4400

RECOMMENDED STORAGE: Airtight container at room temperature.

CHEMICAL/PHYSICAL PROPERTIES: Water-insoluble surfactant with hydrophilic/lipophilic balance (HLB) of approximately 1.0 which classifies the compound as a spreading agent.

INCOMPATIBILITY: None found.

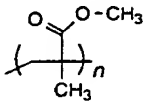
SAFETY: Currently under study.

ADJUVANT PROPERTIES: The amphipathic structure is hypothesized to enhance the presentation of antigen to cells of the immune system. Also see references for SAF-1 and Antigen Formulation.

- Allison, A. C., and Byars, N. E., 1992, Syntex Adjuvant Formulation, *Res. Immunol.* **143**:519–525.
- Hunter, R. L., *et al.*, 1981, The adjuvant activity of nonionic block polymer surfactants I. The role of hydrophile–lipophile balance, *J. Immunol.* **127**:1244–1250.
- Hunter, R. L., *et al.*, 1984, The adjuvant activity of nonionic block polymer surfactants II. Antibody formation and inflammation related to the structure of triblock and octablock copolymers, *J. Immunol.* **133**:3167–3175.
- Hunter, R. L., *et al.*, 1986, The adjuvant activity of nonionic block polymer surfactants III. Characterization of selected biologically active surfaces, *Scand. J. Immunol.* **23**:287–300.
- Lidgate, D. M., and Byars, N., Chapter 12, this volume.

CONTACT(S): Thomas Ryskamp, IDEC Pharmaceuticals Corporation, San Diego, CA 92121, Ph: 619-550-8500; Fax: 619-550-8750; Internet: tryskamp@idec.com. Also: Deborah M. Lidgate, Syntex Corp., Palo Alto, CA 94304, Ph: 415-852-1887; Fax: 415-852-1784.

COMPONENT/ADJUVANT NAME: PMMA
OTHER NAME(S): Polymethyl methacrylate
STRUCTURE:



SOURCE: Emulsion polymerization of methyl methacrylate.

USES: Primary adjuvant for all types of antigens. Added to the aqueous antigens in concentrations of 0.05% to 1.0% (w/w). Optimal adjuvant concentration in most cases 0.5%.

APPEARANCE: White odorless powder; forms a white milky suspension in water.

MOLECULAR WEIGHT: 30,000–400,000, depending on polymerization conditions.

RECOMMENDED STORAGE: Room temperature in solid powder form; between 2 and 8°C in aqueous suspension (pH range 2–11).

CHEMICAL/PHYSICAL PROPERTIES: Insoluble polymer, suspendable in aqueous solution. Forms a milky suspension on dispersion in water, easy to resuspend once hydrated. Polymer particle size 100–500 nm.

INCOMPATIBILITY: None found.

SAFETY/TOXICITY: PMMA has been used as an artificial bone material and bone cement in humans for over 50 years. Breakdown of these artificial bone materials leads to fragments with similar particle size to that of the adjuvant; no adverse reactions have been observed.

ADJUVANT PROPERTIES: Good adsorbate for a large number of antigens, particularly hydrophobic antigens. Antigen may be absorbed to previously polymerized particles, or may be incorporated into the polymer particles by polymerization in the presence of the antigen. PMMA is slowly biodegradable (40%/year in rats). PMMA enhances the temperature stability of a number of antigens.

- Kreuter, J., 1992. Physicochemical characterization of nanoparticles and their potential for vaccine preparation. *Vaccine Res.* 1:93–98.
- Kreuter, J., *et al.*, 1981. Long-term studies of microcapsulated and adsorbed influenza vaccine nanoparticles. *J. Pharm. Sci.* **70**:367–371.
- Kreuter, J., Chapter 19, this volume.

CONTACT(S): Dr. Jorg Kreuter, Institut für Pharmazeutische Technologie, J. W. Goethe-Universität, D-60439 Frankfurt, Germany, Ph: 49-69-5800-9682; Fax: 49-69-5800-9694.

COMPONENT/ADJUVANT NAME: PODDS™

OTHER NAME(S): Proteinoid microspheres

STRUCTURE: Acylated amino acids (early experiments done with thermally condensed α-amino acids).

SOURCE: Chemical synthesis, purified by reprecipitation in acid. Microspheres are made in citric or acetic acid; compounds such as gum arabic, gelatin, or lactose may be added to formulate the material.

USES: Microspheres are being used as vehicles for oral immunization for the development of both mucosal and humoral responses. They are thought to protect antigens, target them to Peyer's patches, and/or facilitate transport of the protein antigens across mucosal epithelium.

APPEARANCE: Exists as liquid suspension or free flowing powder (after lyophilization).

MOLECULAR WEIGHT: 250–300

RECOMMENDED STORAGE: If lyophilized, store at room temperature under low humidity. Stability studies of suspension have not been conducted.

CHEMICAL/PHYSICAL PROPERTIES: Proteinoids have good water solubility at neutral pH, and precipitate out as microspheres at pH 2–3 at concentrations from 20 to 100 mg/mL. Particle size distribution of microspheres ranges from 0.1 to 10 μm, depending on the composition and formulation. Microspheres remain stable at acid pHs, soluble at neutral pHs. Proteinoids interact noncovalently with proteins, and have high encapsulation affinities.

INCOMPATIBILITY: None found.

SAFETY/TOXICITY: Toxicology data available on thermal condensate products in rats and dogs, following acute and subacute i.v. and p.o. dosing; dosing of human volunteers with the thermal condensate carrier resulted in good safety profile. Formal toxicology not completed with acylated amino acid microspheres; these carriers have been administered orally and intraduodenally to rodents and primates at doses up to 1000 mg/kg.

ADJUVANT PROPERTIES: Serves as a vehicle for oral immunization, protecting the antigen and allowing for co-encapsulation of adjuvants with antigens in microspheres.

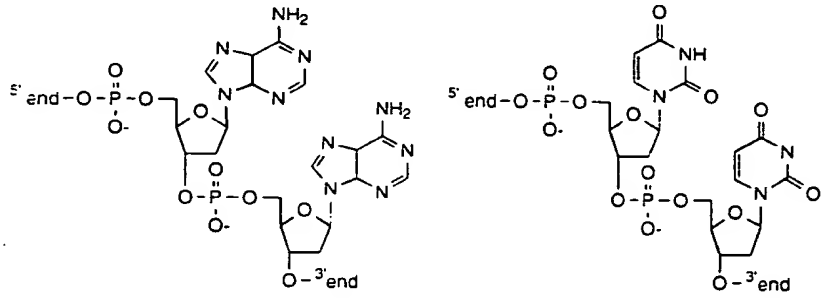
- Santiago, N., *et al.*, 1993. Oral immunization of rats with proteinoid microspheres encapsulating influenza virus antigens. *Pharm. Res.* **10**:1243–1247.
- Santiago, N., *et al.*, Chapter 17, this volume.

CONTACT(S): Noemi Santiago or Robert Baughman, Emisphere Technologies, Inc., Hawthorne, NY 10532, Ph: 914-347-2220; Fax: 914-347-2498.

COMPONENT/ADJUVANT NAME: Poly rA: Poly rU

OTHER NAME(S): Polyadenylic acid-polyuridylic acid complex

STRUCTURE: Poly rA:poly rU is a double helix comprised of polyadenylic acid (left structure, two repeat units shown) and polyuridylic acid (right structure, two repeat units shown).



SOURCE: Synthetic. Polyribonucleotide complexes are formed following the action of the enzyme polynucleotide phosphorylase on the synthetic mononucleotide diphosphate. A hydrogen-bonded double helix forms following mixing of the opposite base pairs.

USES: Immunomodulation.

APPEARANCE: White, odorless powder.

MOLECULAR WEIGHT: Variable, ranging from 200,000 to 2,000,000. Sw_{20} values range from 4 to 11 units.

RECOMMENDED STORAGE: Stable for several years in sterile physiological saline at 4°C.

CHEMICAL/PHYSICAL PROPERTIES: Polyadenylate as potassium salt; polyuridylate as ammonium salt; readily water soluble at neutral pH (pH 7.2–7.6).

INCOMPATIBILITY: Destroyed by RNase.

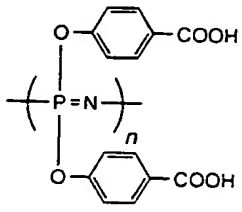
SAFETY/TOXICITY: No toxicity in human trials at 600 mg/m^2/wk for 6 wks.

ADJUVANT PROPERTIES: Adjuvant to humoral and cell-mediated immunity when given with antigen; increases nonspecific immunity to microorganisms; antibody suppressant when given before antigen.

- Johnson, A. G., *et al.*, 1979, Modulation of the immune system by synthetic polynucleotides, *Springer Semin. Immunopathol.* 2:149–168.
- Tursz, T. A., *et al.*, 1990, Poly A-poly U: An updated review, in: *Immunotherapeutic Prospects of Infectious Diseases* (K. N. Mashihi and W. Lange, eds.), Springer-Verlag, Berlin, pp. 263–272.
- Lacour, J., *et al.*, 1984, Adjuvant treatment with polyadenylic-polyuridylic acid in operable breast cancer: update results of a randomized trial, *Br. Med. J.* **288**:589–592.

CONTACT(S): Cynthia Ewel, Institute Henri Beaufour-USA, Washington, DC 20037, Ph: 202-973-2400; Fax: 202-887-5032. Also: A. G. Johnson, University of Minnesota, Duluth, MN 55812, Ph: 218-726-7561; Fax: 218-726-6235.

COMPONENT/ADJUVANT NAME: Polyphosphazene
OTHER NAME(S): PCPP; poly[di(carboxylatophenoxy)]phosphazene
STRUCTURE:



SOURCE: Synthetic.

USES: As an adjuvant for parenteral formulations. As a microsphere hydrogel for mucosal formulations.

APPEARANCE: White, odorless substance.

MOLECULAR WEIGHT: 3000–10,000,000

RECOMMENDED STORAGE: Store at a temperature not exceeding 30°C.

CHEMICAL/PHYSICAL PROPERTIES: Soluble in aqueous alkali solutions; ionically cross-linkable in aqueous media when treated with salts of di- or trivalent cations; T_g = –4.7°C.

INCOMPATIBILITY: None found.

SAFETY/TOXICITY: Subcutaneous injection of polyphosphazine in mice showed an intense inflammatory response without formation of a granuloma.

ADJUVANT PROPERTIES: Induces a sustained antibody response in mice after a single parenteral dose. High functional antibody titers are induced. The response is largely IgG1. Sustained IgG and IgA responses are also induced in mice after mucosal immunization.

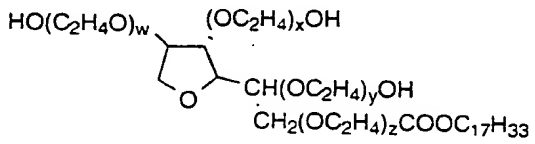
- Payne, L. G., *et al.*, Chapter 20, this volume.

CONTACT(S): Lendon G. Payne, Virus Research Institute, Cambridge, MA 02134, Ph: 617-864-6232; Fax: 617-864-6334.

COMPONENT/ADJUVANT NAME: Polysorbate 80

OTHER NAME(S): Tween 80; Sorbitan mono-9-octadecenoate poly(oxy-1,2-ethanediyl) derivatives

STRUCTURE:



SOURCE: Polysorbate 80 is produced via copolymerization of ethylene oxide with an oleate ester of sorbitan and its anhydrides.

USES: A stabilizer in the MF59 and IDEC SPT formulations, present in final concentration of approximately 0.2% (w/v) with antigen. Commonly used surfactant in foods, cosmetics, and pharmaceuticals.

APPEARANCE: Amber, viscous liquid.

MOLECULAR WEIGHT: 1309.68

RECOMMENDED STORAGE: Airtight container at room temperature.

CHEMICAL/PHYSICAL PROPERTIES: HLB of 12–16 and therefore highly soluble in aqueous solution. The oleic acid esters are susceptible to oxidation.

INCOMPATIBILITY: Avoid strong oxidizing agents, bases, and heavy metal salts.

SAFETY/TOXICITY: Mild ocular irritant (rabbit eye test 150 mg). LD_{50} (rat) via i.v., 1.8 g/kg; LD_{50} (mouse) via oral, 25 g/kg. Generally considered safe (GRAS).

ADJUVANT PROPERTIES: Polysorbate 80 has no adjuvant properties on its own. Used in emulsion vaccine formulations including MF59, SAF-1, and Antigen Formulation. See those headings for additional references.

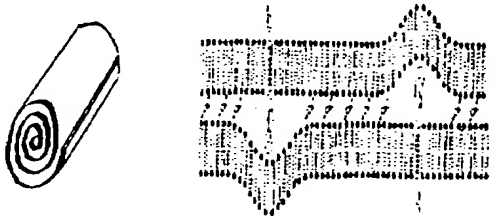
- Sanchez-Pestador, L., *et al.*, 1988. The effect of adjuvants on the efficacy of a recombinant herpes simplex glycoprotein vaccine. *J. Immunol.* **141**:1720–1727.
- Van Nest, G. A., *et al.*, 1992. Advanced adjuvant formulations for use with recombinant subunit vaccines, in: *Vaccines 92* (F. Brown, R. M. Chanock, H. S. Ginsberg, and R. A. Lerner, eds.), Cold Spring Harbor Press, Cold Spring Harbor, NY, p. 57.
- Ott, G., *et al.*, Chapter 10, this volume.
- Lidgate, D. M., and Byars, N., Chapter 12, this volume.

CONTACT(S): There are several suppliers of Polysorbate 80. For use in adjuvant formulations: Thomas Ryskamp, IDEC Pharmaceuticals Corporation, San Diego, CA 92121, Ph: 619-550-8500; Fax: 619-550-8750; Internet: tryskamp@idec.com. Also: Gary Van Nest, Chiron Corp., Emeryville, CA, Ph: 510-601-2965; Fax: 510-601-2586. Also: Deborah M. Lidgate, Syntex Research, Palo Alto, CA 94304, Ph: 415-852-1887; Fax: 415-852-1784.

COMPONENT/ADJUVANT NAME: Protein Cochleates

OTHER NAME(S): None

STRUCTURE: Protein cochleates are stable protein phospholipid–calcium precipitates, which are distinct from liposomes. They have a unique structure consisting of a large, continuous, solid, lipid bilayer sheet rolled up in a spiral, with no internal aqueous space. The calcium maintains the cochleate in its rolled-up form, bridging between successive layers. One of its positive charges interacts with a single negative charge on a phospholipid head group in one bilayer, and the other with a phospholipid in the opposing bilayer. Membrane proteins, or lipid-anchored peptides or proteins are tightly associated with the lipid bilayer.



SOURCE: Cholesterol, phosphatidylethanolamine (egg or synthetic), and phosphatidylserine (bovine brain or synthetic) are obtained from Avanti Polar Lipids, Inc. Antigens which have been utilized include glycoproteins isolated directly from enveloped viruses, or expressed as recombinants in tissue culture, as well as synthetic peptides covalently linked to phosphatidylethanolamine. A mixture of phospholipids from the envelope will also be included when glycoproteins are isolated from viruses by our method of detergent extraction.

USES: Protein cochleates act as both carriers and adjuvants, providing multivalent presentation of antigens to the immune system, with maintenance of native conformation and biological activity. Protection of antigens from degradation following oral delivery. Probable controlled or slow release properties.

APPEARANCE: White, fine-grained suspension or precipitate. May be lyophilized to a white powder.

MOLECULAR WEIGHT: Macromolecular structure of varying size depending on antigen and lipid content.

RECOMMENDED STORAGE: Protein cochleates are stable for at least 6 months at 4°C. Alternatively, they may be lyophilized and stored at room temperature for 6 months as a powder, and reconstituted with liquid prior to administration. Storage for longer time periods and higher temperatures has not been assessed.

CHEMICAL/PHYSICAL PROPERTIES: Protein cochleates are formed at or near neutral pH. Their stability to extremes of pH has not been characterized, but they are capable of protecting associated antigens when given orally. This is probably related to their unique rolled-up solid precipitate structure which prevents the exposure of antigens within the interior of the spiral to the external milieu.

INCOMPATIBILITY: None found.

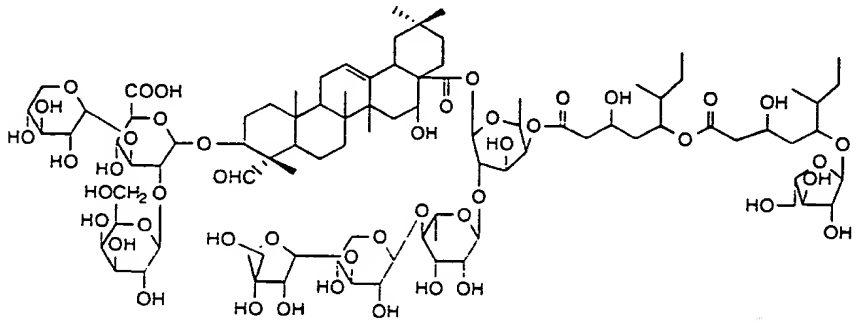
SAFETY/TOXICITY: The phospholipids used in the preparation of protein cochleates have been used in humans for vaccines and drug delivery with no significant negative side effects. Protein cochleates have been given to hundreds of mice by various routes including oral, intramuscular, and intranasal, with no negative local or systemic effects noted.

ADJUVANT PROPERTIES: Protein cochleates stimulate strong mucosal and systemic antibody, proliferative, and cytotoxic responses to associated antigens. They also afford protection from degradation following oral delivery and probable slow release properties.

- Gould-Fogerite, S., and Mannino, R. J., 1992, Targeted fusogenic liposomes: Functional reconstitution of membrane proteins into large unilamellar vesicles via protein-cochleate intermediates, in: *Liposome Technology* 2nd ed. (G. Gregoriadis, ed.), CRC Press, Boca Raton, Vol. III, pp. 262–275.
- Gould-Fogerite, S., *et al.*, 1994, Orally delivered protein cochleate vaccines stimulate mucosal and circulating immunity and protection from mucosal challenge with live influenza virus, (submitted).
- Mannino, R. J., and Gould-Fogerite, S., Chapter 15, this volume.

CONTACT(S): Dr. Susan Gould-Fogerite and Dr. Raphael J. Mannino, MuDNJ, New Jersey Medical School, Dept. of Laboratory Medicine and Pathology, Newark, NJ 07103-2714, Ph: 201-982-7836; Fax: 201-982-7293.

COMPONENT/ADJUVANT NAME: QS-21
OTHER NAME(S): Stimulon™ QS-21 Adjuvant
STRUCTURE:



SOURCE: Natural product of the bark of the *Quillaja saponaria* Molina tree (species native to Chile and Argentina). Extracted from the bark by aqueous extraction. Purified by normal phase and reverse phase chromatography.

- Kensil, C. R., *et al.*, 1991, Separation and characterization of saponins with adjuvant activity from *Quillaja saponaria* Molina cortex, *J. Immunol.* **146**:431–437.

USES: Used in vaccine formulations as a primary adjuvant component for enhancement of both humoral and cell-mediated immunity. Water soluble. No emulsification required. Can be used alone or combined with aluminum hydroxide adjuvant.

APPEARANCE: Solid: white, odorless powder. Aqueous solution: clear, colorless solution.

MOLECULAR WEIGHT: Parent: 1990, sodium salt: 2012.

RECOMMENDED STORAGE: Store solid QS-21 under low-humidity conditions at −20°C. Protect from light. Optimum storage conditions are under evaluation. No apparent degradation under low-humidity conditions after storage at 25°C for 8 weeks. Aqueous solutions are optimally stable between pH 5 and 6 and in micellar form. Solutions of QS-21 in 0.5 mg/mL solution may be stored in this pH range at 5°C for 2 or 3 years. Protect from light. In aqueous solution, the fatty acid ester bond migrates between the 3 and 4 position on fucose, with the ester at the 4 position being favored. Both forms are active as adjuvants. Primary degradation reaction is alkaline hydrolysis of the fatty acid ester bond at the 3 or 4 position on fucose. Due to alkaline-catalyzed degradation reaction, sterilization should be carried out by membrane filtration instead of autoclaving.

CHEMICAL/PHYSICAL PROPERTIES: Amphiphilic molecule with good water solubility above pKa of carboxyl group (solubility approximately 2 mg/mL to pH 4, 15 mg/mL at pH 5, 28 mg/mL at pH 6, and 32 mg/mL at pH 7 in buffered saline solutions). Forms micelles in aqueous solution (cmc approximately 50 μg/mL in phosphate-buffered saline, pH 7.0).

INCOMPATIBILITY: Avoid strong bases.

SAFETY/TOXICITY: Studies in human Phase I clinical trial of therapeutic melanoma vaccine at doses up to 200 μg by subcutaneous route. Currently under study in human Phase I clinical trial of prophylactic HIV-1 subunit vaccine at doses up to 100 μg by intramuscular route.

- Livingston, P. O., *et al.*, 1994, Phase I trial of immunological adjuvant QS-21 with a GM2 ganglioside-KLH conjugate vaccine in patients with malignant melanoma, *Vaccine* **12(14)**:1275–1280.

ADJUVANT PROPERTIES: Shown to stimulate humoral immune responses in mice, including antigen-specific IgG1, IgG2b, and IgG2a titers. Augments production of IgG responses to ganglioside antigen in melanoma vaccine in human Phase I clinical trials. Shown also to stimulate CTL responses in mice.

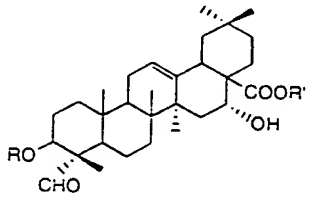
- Kensil, C. R., *et al.*, 1993, The use of Stimulon adjuvant to boost vaccine response, *Vaccine Res.* **2(4)**:273–281.
- Newman, M. J., *et al.*, 1992, Saponin adjuvant induction of ovalbumin specific CD8+ cytotoxic T-lymphocyte responses, *J. Immunol.* **138**:2357–2362.
- Augments production of IgG responses to ganglioside antigen in melanoma vaccine in human Phase I clinical trial (Livingston, P. O., 1993, Approaches to augmenting the IgG antibody response to melanoma ganglioside vaccines, *Ann. N.Y. Acad. Sci.* **690**:204–213.
- Kensil, C., *et al.*, Chapter 22, this volume.
- Coughlin, R. T., *et al.*, Chapter 32, this volume.

CONTACT(S): Dr. Edward Balkovic, Cambridge Biotech Corporation, Worcester, MA 01605, Ph: 508-797-5777; Fax: 508-797-4014.

COMPONENT/ADJUVANT NAME: Quil A

OTHER NAME(S): Quil A saponin, *Quillaja* saponin

STRUCTURE: A complex but purified mixture of *Quillaja* saponins which are glycosides of quillaic acid and carbohydrates. The aglycone of Quil A is shown below.



SOURCE: Purified extract from the bark of the South American tree *Quillaja saponaria* Molina.

USES: Quil A is used in veterinary vaccines and for production of ISCOMs.

APPEARANCE: Lyophilized powder. Color is light brownish, almost white.

MOLECULAR WEIGHT: Ranges from approximately 1400 to 2400

RECOMMENDED STORAGE: Dry storage in the lyophilized state. Can be stored frozen, refrigerated, or at room temperature.

CHEMICAL/PHYSICAL PROPERTIES: The mixture contains fractions that bind to cholesterol, are adjuvant active, are hemolytic, and are able to form ISCOMs.

INCOMPATIBILITY: Should not be exposed to alkaline conditions (pH > 8.0).

SAFETY/TOXICITY: Avoid inhalation and eye contact when handling Quil A. It is highly irritating to mucosa and contains hemolyzing saponins. Quil A is not used in human trials because of overt toxicity. It is, however, used extensively in veterinary vaccines.

- Speijers, G. J. A., *et al.*, 1988. Local reactions of the saponin Quil A and a Quil A containing iscom measles vaccine after intramuscular injection of rats: A comparison with the effects of DTP-polio vaccine. *Fundam. Appl. Toxicol.* **10**:425–430.

ADJUVANT PROPERTIES: Quil A is used as a part of a novel antigen presentation system called ISCOMs, as well as with antigen alone. It induces both humoral and cell-mediated responses.

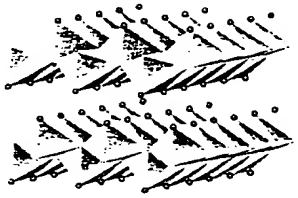
- Morein, B., 1984. Iscom, a novel structure for antigenic presentation of membrane proteins from enveloped viruses. *Nature* **308**:457–460.
- Dalsgaard, K., *et al.*, 1977. Evaluation of the adjuvant "Quil-A" in the vaccination of cattle against foot-and-mouth disease. *Acta Vet. Scand.* **18**:349–360.
- Dalsgaard, K., 1984. Assessment of the dose of the immunological adjuvant Quil-A in mice and guinea pigs, using sheep red blood cells as model antigen. *Zbl. Vet. Med. B* **31**:718–720.
- Dalsgaard, K., and Jensen, M. H., 1977. The adjuvant activity of "Quil-A" in trivalent vaccination of cattle and guinea pigs against foot-and-mouth disease. *Acta Vet. Scand.* **18**:367–373.
- Rimmelzwaan, G. F., and Osterhaus, A. D. M. E., Chapter 23, this volume.

CONTACT(S): There are several suppliers of Quil A. The monograph prepared by: E. B. Lindblad, Superfos Biosector, DK-2950 Vedbaek, Denmark, Ph: 45 42 89 31 11; Fax: 45 42 89 15 95. Also: Al Reisch, Sergeant, Inc., Clifton, NJ 07012, Ph: 201-472-9111; Fax: 201-472-5686. Also: Accurate Chemical & Scientific Corp., Westbury, NY 11590, Ph: 800-645-6264; Fax: 516-997-4948.

COMPONENT/ADJUVANT NAME: Rehydragel HPA

OTHER NAME(S): High protein adsorbency aluminum hydroxide gel; alum

STRUCTURE: Crystalline aluminum oxyhydroxide AlOOH, known mineralogically as boehmite. The structure consists of corrugated sheets of aluminum octahedra.



SOURCE: Synthetic oxyhydroxide of aluminum (aluminum hydroxide) prepared by acid–base precipitation.

USES: Primary adjuvant in parenteral vaccine formulations. Does not generally induce cell-mediated immunity.

APPEARANCE: Translucent, thixotropic, colloidal aqueous gel supplied sterile.

MOLECULAR WEIGHT: 60 (empirical formula)

RECOMMENDED STORAGE: Stable at room temperature for indefinite period. Freezing should be avoided.

CHEMICAL/PHYSICAL PROPERTIES: Contains 2% equivalent Al_2O_3 or 3% equivalent $Al(OH)_3$. Primary particles have a rodlike morphology and a high surface area. The isoelectric point is 11. Its high surface area gives it a high adsorptive capacity for antigens. Typical pH: 5.8 to 6.8. Insoluble in water between pH 4 and 8, and poorly soluble in solutions containing citrate ion. Average particle size: 0.3 μm. Pumpable suspension.

INCOMPATIBILITY: Do not freeze, otherwise chemically inert and stable.

SAFETY/TOXICITY: Aluminum compounds (aluminum hydroxide, aluminum phosphate, alum) are currently the only vaccine adjuvants used in U.S.-licensed vaccines. They can induce granulomas at the inoculation site. Supplied pyrogen free.

- Ganrot, P. O., 1986, Metabolism and possible health effects of aluminum, *Environ. Health Perspect.* **65**:363–441.
- Gupta, R. K., *et al.*, 1993, Adjuvants—A balance between toxicity and adjuvanticity, *Vaccine* **11**:293–306.

ADJUVANT PROPERTIES: Protein binding capacity: 2.5 mg BSA/mg Al_2O_3 minimum. The surface area, surface charge, and morphology of the aluminum hydroxide are major factors in its adjuvant characteristics. The use of aluminum adjuvants is accompanied by stimulation of IL-4 and stimulation of the T-helper-2 subsets in mice, with enhanced IgG1 and IgE production.

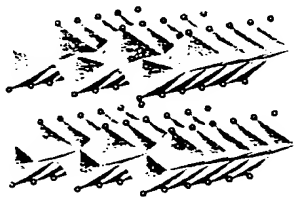
- Shirodkar, S., *et al.*, 1990, Aluminum compounds used as adjuvant in vaccines, *Pharm. Res.* **7**:1282–1288.
- Aprile, M. A., and Wardlaw, A. C., 1966, Aluminum compounds as adjuvants for vaccines and toxoids in man, *Can. J. Public Health* **57**:343–354.
- Gupta, R. K., *et al.*, Chapter 8, this volume.
- Seeber, S., *et al.*, 1991, Predicting the adsorption of proteins by aluminum-containing adjuvants, *Vaccine* **9**:201–203.
- Seeber, S. J., *et al.*, 1991, Solubilization of aluminum-containing adjuvants by constituents of interstitial fluid, *J. Parenteral Sci. Tech.* **45**:156–159.
- Hem, S., and White, J. L., Chapter 9, this volume.

CONTACT(S): Philip B. Klepak, Reheis Inc., Berkeley Heights, NJ 07922, Ph: 908-464-1500; Fax: 908-464-7726. Also: Stanley Hem, Purdue University, West Lafayette, IN 47907-1336, Ph: 317-494-1451; Fax: 317-494-7880.

COMPONENT/ADJUVANT NAME: Rehydragel LV

OTHER NAME(S): Low-viscosity aluminum hydroxide gel; alum

STRUCTURE: Crystalline aluminum oxyhydroxide AlOOH, known mineralogically as boehmite. The structure consists of corrugated sheets of aluminum octahedra.



SOURCE: Synthetic oxyhydroxide of aluminum (aluminum hydroxide) prepared by acid–base precipitation.

USES: Primary adjuvant in parenteral vaccine formulations. Does not generally induce cell-mediated immunity.

APPEARANCE: White, fluid aqueous suspension supplied sterile.

MOLECULAR WEIGHT: 60 (empirical formula)

RECOMMENDED STORAGE: Stable at room temperature for indefinite period. Freezing should be avoided.

CHEMICAL/PHYSICAL PROPERTIES: Contains 2% equivalent Al_2O_3 or 3% equivalent $Al(OH)_3$. Primary particles have a rodlike or fibril morphology, but are larger than Rehydragel HPA. Surface area and antigen absorptive capacity diminished compared with Rehydragel HPA. Typical pH is 5.8 to 6.8. Insoluble in water between pH 4 and 8, and poorly soluble in solutions containing citrate ion. Average particle size: 1 μm. Has a low viscosity and is pumpable.

INCOMPATIBILITY: Do not freeze, otherwise chemically inert and stable.

SAFETY/TOXICITY: Aluminum compounds (aluminum hydroxide, aluminum phosphate, alum) are currently the only vaccine adjuvants used in U.S.-licensed vaccines. They can induce granulomas at the inoculation site. Supplied pyrogen free.

- Ganrot, P. O., 1986, Metabolism and possible health effects of aluminum, *Environ. Health Perspect.* **65**:363–441.
- Gupta, R. K., *et al.*, 1993, Adjuvants—A balance between toxicity and adjuvanticity, *Vaccine* **11**:293–306.

ADJUVANT PROPERTIES: Protein binding capacity: 1.5 mg BSA/mg equivalent Al_2O_3 minimum. The surface area, surface charge, and morphology are major factors in its adjuvant characteristics. The use of aluminum adjuvants is accompanied by stimulation of IL-4 and stimulation of the T-helper-2 subsets in mice, with enhanced IgG1 and IgE production.

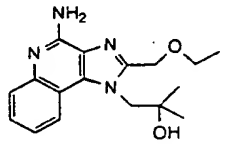
- Aprile, M. A., and Wardlaw, A. C., 1966, Aluminum compounds as adjuvants for vaccines and toxoids in man, *Can. J.* [illegible]
- Gupta, R. K., *et al.*, Chapter 8, this volume.
- Seeber, S., *et al.*, 1991, Predicting the adsorption of proteins by aluminum-containing adjuvants, *Vaccine* **9**:201–203.
- Seeber, S. J., *et al.*, 1991, Solubilization of aluminum-containing adjuvants by constituents of interstitial fluid, *J. Parenteral Sci. Tech.* **45**:156–159.
- Hem, S., and White, J. L., Chapter 9, this volume.

CONTACT(S): Philip B. Klepak, Reheis Inc., Berkeley Heights, NJ 07922, Ph: 908-464-1500; Fax: 908-464-7726. Also: Stanley Hem, Purdue University, West Lafayette, IN 47907-1336, Ph: 317-494-1451; Fax: 317-494-7880.

COMPONENT/ADJUVANT NAME: S-28463

OTHER NAME(S): 4-Amino-α,α-dimethyl-2-ethoxymethyl-1*H*-imidazo[4,5-*c*]quinoline-1-ethanol

STRUCTURE:



SOURCE: Chemical synthesis. International Publication 92/15582.

USES: Included in adjuvant formulations as a primary adjuvant component.

APPEARANCE: White, fine crystalline solid.

MOLECULAR WEIGHT: 314.39 free base, 350.85 hydrochloride salt.

RECOMMENDED STORAGE: Solid is stable at room temperature. Shelf life is acceptable.

CHEMICAL/PHYSICAL PROPERTIES: Somewhat limited solubility as the free base. The hydrochloride salt is soluble in water at concentrations at least to 10 mg/mL.

INCOMPATIBILITY: None found.

SAFETY/TOXICITY: In preclinical animal safety evaluation studies.

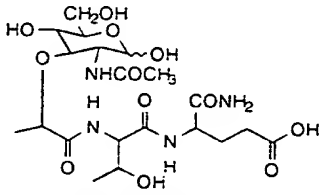
ADJUVANT PROPERTIES: Addition of S-28463 induces both humoral and cell-mediated immunity via induction of cytokines from monocytes and macrophages. Unpublished results indicate S-28463 is about 100-fold more potent than imiquimod in antiviral models and in cytokine induction from monocytes and macrophages.

CONTACT(S): R. C. Hanson, Business Development, 3M Pharmaceuticals, St. Paul, MN 55144, Ph: 612-737-3137; Fax: 612-737-4556.

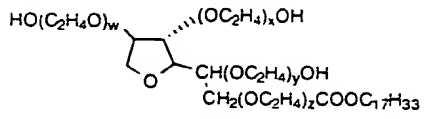
COMPONENT/ADJUVANT NAME: SAF-1

OTHER NAME(S): SAF, SAF-m; Syntex Adjuvant Formulation

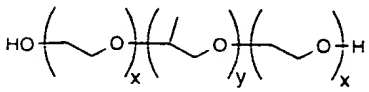
STRUCTURE: Composed of threonyl-MDP (0.05–~1%) in an emulsion vehicle [5% squalane, 2.5% Pluronic® L121, 0.2% Polysorbate 80, and phosphate-buffered saline (pH 7.4)].



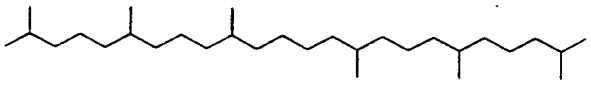
Threonyl-MDP



Polysorbate 80



Pluronic L121



Squalane

SOURCE: See individual components.

USES: Adjuvant formulation.

APPEARANCE: White, fluid, oil-in-water emulsion.

MOLECULAR WEIGHT: Not applicable (see individual components).

RECOMMENDED STORAGE: ≤30°C.

CHEMICAL/PHYSICAL PROPERTIES: Particle size depends on the manufacturing method used. If the emulsion is manufactured using a Microfluidizer, then the mean particle size is ~160 nm.

INCOMPATIBILITY: None found.

SAFETY/TOXICITY: At therapeutic doses, no safety concern is anticipated. Dose is indication dependent, but a typical volume of injection is 1 mL.

ADJUVANT PROPERTIES: Antigens become arranged on the surface of the emulsion droplets partly because of their amphipathic nature, and partly because of hydrogen bonding with poloxamer 401. The emulsion droplets also activate complement, as demonstrated by consumption of C3 and production of C3b; the latter, on the surface of droplets, targets them to antigen-presenting cells (follicular dendritic cells and interdigitating cells) in lymph nodes of the drainage chain and possibly in more distant lymphoid tissues. In this way the emulsion facilitates the presentation of antigens to responding lymphocytes. See threonyl-MDP monograph.

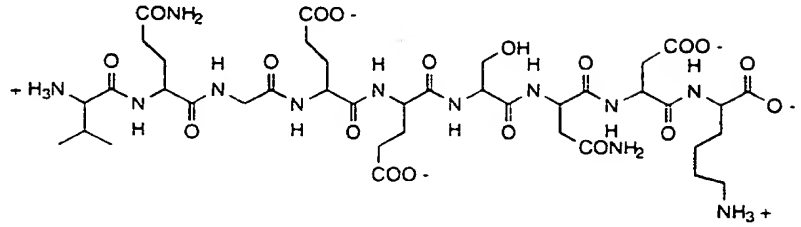
- Allison, A. C., and Byars, N. E., 1992, Syntex Adjuvant Formulation, *Res. Immunol.* **143**:519–525.
- Byars, N. E., and Allison, A. C., 1987, Adjuvant formulation for use in vaccine to elicit both cell mediated and humoral immunity, *Vaccine* 5:223–228.
- Allison, A. C., *et al.*, 1986, An adjuvant formulation that selectively elicits the formation of antibodies of protective isotypes and of cell-mediated immunity, *J. Immunol. Methods* **95**:157–168.
- Lidgate, D. M., and Byars, N., Chapter 12, this volume.

CONTACT(S): Deborah M. Lidgate, Syntex Research, Palo Alto, CA 94304, Ph: 415-852-1887; Fax: 415-852-1784.

COMPONENT/ADJUVANT NAME: Sclavo Peptide

OTHER NAME(S): IL-1β 163–171 peptide

STRUCTURE: VQGEESNDK •HCl



SOURCE: From human IL-1β amino acid sequence. Obtained by solid phase synthesis, purified by HPLC and ion-exchanged to the HCl salt.

USES: Primary adjuvant. Active either when administered separately from antigen, or admixed with antigen, or physically linked to antigen. Routes of administration: i.v., i.p., s.c., p.o.

APPEARANCE: White, odorless powder.

MOLECULAR WEIGHT: 1000

RECOMMENDED STORAGE: Stored lyophilized peptide dry at –20°C. Stable also at room temperature. The concentrated solution can be stored in siliconized glass at 4°C for at least 2–3 months. Do not freeze.

CHEMICAL/PHYSICAL PROPERTIES: Good solubility in water. Very acidic. Adjust pH to neutrality before use.

INCOMPATIBILITY: Avoid peptidases.

SAFETY/TOXICITY: No toxicity in mice when given i.v. as a bolus up to 100 mg/kg.

ADJUVANT PROPERTIES: It enhances immune response to T-dependent and T-independent antigens. Active also in increasing secondary responses. Active as adjuvant for a tumor vaccine. Antitumor activity through recruitment of host immune response.

- Nencioni, L., *et al.*, 1987. In vivo immunostimulating activity of the 163–171 peptide of human IL-1β, *J. Immunol.* **139**:800–804.
- Boraschi, D., *et al.*, 1988. In vivo stimulation and restoration of the immune response by the noninflammatory fragment 163–171 of human IL-1β. *J. Exp. Med.* **168**:675–686.
- McCune, C. S., and Marquis, D. M., 1990. Interleukin-1 as an adjuvant for active specific immunotherapy in a murine tumor model. *Cancer Res.* **50**:1212–1215.
- Rao, K. V. S., and Nayak, A. R., 1990. Enhanced immunogenicity of a sequence derived from hepatitis B virus surface antigen in a composite peptide which includes the immunostimulatory region from human interleukin-1. *Proc. Natl. Acad. Sci. USA* **87**:5519–5522.
- Beckers, W., *et al.*, 1993. Increasing the immunogenicity of protein antigens through the genetic insertion of VQGEESNDK sequence of human IL-1β into their sequence, *J. Immunol.* **151**:1757–1764.

CONTACT(S): Dr. P. Ghiara, IRIS-Biocine, Siena, Italy, Ph: 39-577-293111; Fax: 39-577-293564. Also: Drs. A. Tagliabue and D. Boraschi, Dompè Research Center, L'Aquila, Italy, Ph: 39-862-338324; Fax: 39-862-338219.

COMPONENT/ADJUVANT NAME: Sendai Proteoliposomes
Sendai-Containing Lipid Matrices

OTHER NAME(S): Sendai glycoprotein-containing vesicles; fusogenic proteoliposomes; FPLs; Sendai lipid matrix-based vaccines

STRUCTURE: Sendai proteoliposome: The glycoproteins of Sendai virus (parainfluenza type 1) are integrated in the lipid bilayers of large, mainly unilamellar, liposomes. Native conformation and biological activities of receptor binding and membrane fusion are maintained. Other proteins containing hydrophobic regions or lipid-anchored proteins or peptides may be encapsulated in the lipid bilayer. Water-soluble proteins or other materials may be encapsulated in the aqueous interior of the vesicles.

Sendai-containing lipid matrices: Some peptides which are amphipathic (i.e., possess both hydrophilic and hydrophobic regions) have the ability to collapse lipid bilayers. When these peptides are encapsulated by adding EDTA to Sendai protein cochleates, lipid aggregates, rather than liposomes (with a continuous lipid bilayer encapsulating an internal aqueous space), are produced. Polymorphic lipid aggregates also form when plain lipid cochleates are converted by EDTA in the presence of high concentrations of these amphipathic peptides.

SOURCE: Prepared from Sendai protein cochleates by chelation of Ca^{2+} with EDTA. See Protein cochleates for lipids and antigens used and sources. Material encapsulated includes chemically synthesized peptides, isolated and recombinant proteins, whole fixed viruses, small-molecule drugs, and DNA.

USES: Sendai proteoliposomes produced by these methods are highly effective immunogens in mice, rabbits, and monkeys. This includes the ability to stimulate strong T-helper and $CD8^+$ cytotoxic T-cell responses (CTL) to lipid bilayer-integrated glycoproteins as well as encapsulated peptides, proteins, and whole formalin-fixed viruses. These vesicles also act as effective delivery vehicles for drugs and proteins. They were used to achieve the first stable gene transfer in animals using a liposome-based system. These abilities probably arise from their membrane attachment and fusion activity which facilitates introduction into the cytoplasm and access to an MHC class I presentation pathway.

APPEARANCE: Opalescent suspension in aqueous isotonic buffer.

MOLECULAR WEIGHT: Macromolecular structure of varying size depending on antigen and lipid content.

RECOMMENDED STORAGE: Phospholipids used as raw materials are stored in chloroform at 20°C under nitrogen. Proteoliposomes should be stored at 4°C in isotonic buffer. They are generally used within a few days of preparation. Long-term stability has not been assessed.

CHEMICAL/PHYSICAL PROPERTIES: Proteoliposomes are stable in aqueous isotonic buffers. They are solubilized by detergents or organic solvents in sufficient quantities.

INCOMPATIBILITY: None found.

SAFETY/TOXICITY: The phospholipids used in the preparation of proteoliposomes have been used in humans for vaccines and drug delivery with no significant negative side effects. Proteoliposomes have been given to hundreds of mice, by intraperitoneal and intramuscular immunization, and many rabbits and 16 monkeys by intramuscular immunization with no negative local or systemic effects noted.

ADJUVANT PROPERTIES: Proteoliposomes stimulate strong antibody and proliferative responses to associated antigens. They are particularly powerful inducers of cytotoxic T lymphocytes. Antigens can be associated with the lipid bilayer or encapsulated within the aqueous interior.

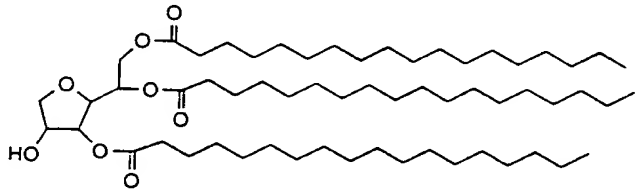
- Gould-Fogerite, S., *et al.*, 1989, Chimerasome-mediated gene transfer in vitro and in vivo, *Gene* **84**:429–438.
- Gould-Fogerite, S., and Mannino, R. J., 1992, Targeted fusogenic liposomes: Functional reconstitution of membrane proteins into large unilamellar vesicles via protein-cochleate intermediates, in: *Liposome Technology*, 2nd ed. (G. Gregoriadis, ed.), CRC Press, Boca Raton, Vol. III, pp. 262–275.
- Miller, M. D., *et al.*, 1992, Vaccination of rhesus monkeys with synthetic peptide in a fusogenic proteoliposome elicits simian immunodeficiency virus-specific $CD8^+$ cytotoxic T lymphocytes, *J. Exp. Med.* **176**:1739–1744.
- Mannino, R. J., and Gould-Fogerite, S., Chapter 15, this volume.

CONTACT(S): Dr. Susan Gould-Fogerite and Dr. Raphael J. Mannino, UMDNJ, New Jersey Medical School, Dept. of Laboratory Medicine and Pathology, Newark, NJ 07103-2714, Ph: 201-982-7836; Fax: 201-982-7293.

COMPONENT/ADJUVANT NAME: Span 85

OTHER NAME(S): Arlacel 85, sorbitan trioleate

STRUCTURE: Spans are partial esters of common fatty acids (lauric, palmitic, stearic, and oleic) and hexitol anhydrides (hexitans and hexides), derived from sorbitol. An example structure is shown below.



SOURCE: Synthetic.

USES: Used as an emulsification agent in MF59 adjuvant formulation.

APPEARANCE: Viscous yellow liquid.

MOLECULAR WEIGHT: Most spans are actually mixtures with one particular span predominating.

RECOMMENDED STORAGE: Store in a cool dry place.

CHEMICAL/PHYSICAL PROPERTIES: Span products tend to be oil-soluble. Span 85 is insoluble in water but can be dispersed with a hydrophilic surfactant. Density 0.956.

INCOMPATIBILITY: Strong oxidizing agents.

SAFETY/TOXICITY: Vapor or mist is irritating to mucous membranes. Causes skin irritation.

ADJUVANT PROPERTIES: None described for the compound itself. See MF59.

- Ott, G., *et al.*, Chapter 10, this volume.

CONTACT(S): Several suppliers offer Span 85. Sigma Chemical Company, Ph: 800-325-3010. For vaccine formulation use: Gary Van Nest, Chiron Corp., Emeryville, CA, Ph: 510-601-2965; Fax: 510-601-2586.

COMPONENT/ADJUVANT NAME: Specol

STRUCTURE(S): Marcol 52 (mineral oil, paraffins, and cycloparaffins, chain length 13-22 C atoms)

Span 85 (emulsifier, sorbitan trioleate)

Tween 85 (emulsifier, polyoxyethylene-20-trioleate)

SOURCE: Ingredients are commercially available and all are individually FDA approved for veterinary use. Mineral oil and emulsifiers (Span and Tween) are thoroughly mixed 9:1 (v/v) and can be stored at 4°C for prolonged periods of time (several years).

USES: Specol can be obtained from ID-DLO in Lelystad and is a primary adjuvant (only antigen needed). The adjuvant mixture of mineral oil and emulsifiers is mixed with the water phase (physiological saline) containing the immunogen (water: oil = 0.44) and emulsified. A stable emulsion is obtained when the second of two drops, deposited on the surface of a water-containing tube, continues to float intact. When a stable (sterile) emulsion is obtained this can be stored for up to 1 year at 4–16°C or 3 months at 37°C (dependent on antigen). It functions as a depot (slow release of antigen) and a polyclonal activator (independent of presence of antigen) for cells of the immune system (cytokine release).

APPEARANCE: Specol is a clear oily fluid. The water-in-oil (w/o) emulsion resulting from mixing Specol with immunogen/water is white and gel-like.

MOLECULAR WEIGHT: Not applicable.

RECOMMENDED STORAGE: 4°C in low-oxygen conditions (e.g., in completely filled bottles or under N_2). As stated below, both Specol and the emulsion are relatively insensitive to temperature changes.

CHEMICAL/PHYSICAL PROPERTIES: The water-in-oil emulsion (Specol emulsified with immunogen in water) is resistant to temperature shifts (4–37°C). It has a low conductivity (indicative of proper separation of oil and water) of <0.6 siemens and a low viscosity (enabling easy application by injection) of 70–100 mPa/s (both measured at 20°C).

INCOMPATIBILITY: The Specol–water/immunogen emulsion is not compatible with natural rubber and is probably incompatible with most organic solvents as is common for w/o emulsions.

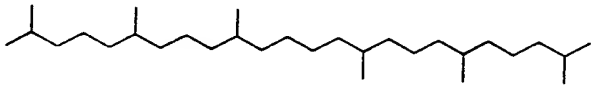
SAFETY: No known use in humans, registered for veterinary use by itself (nonspecific stimulation of immune system, e.g., in weanlings) or in combination with vaccines.

ADJUVANT PROPERTIES: The adjuvant properties of Specol, which are comparable to CFA, in rodents are reviewed in:

- Boersma, W. J. A., *et al.*, 1992, Adjuvant properties of stable water-in-oil emulsions, evaluation of the experience with Specol. 44th Forum in Immunology, *Res. Immunol.* **143**:503–512.
- Bokout, B. A., *et al.*, 1981, A selected water in oil emulsion: Composition and usefulness as an immunological adjuvant, *Vet. Immunol. Immunopathol.* **2**:491–500.

CONTACT(S): Source: Dr. B. Bokout, Institute of Animal Science and Health, POB 65, 8200 AB, The Netherlands, Ph: + 31 3200 73432; Fax: + 31 3200 73473; email: B.A.Bokhout@CDI.AGRO.NL. Rodent studies: Prof. Dr. E. Claassen, TNO-Prevention and Health. Fax + 31 71 181 276.

COMPONENT/ADJUVANT NAME: Squalane
OTHER NAME(S): Spinacane; Robane®; 2,6,10,15,19,23-hexamethyltetracosane
STRUCTURE:



SOURCE: Obtained by the total hydrogenation of the triterpene squalene, a component of shark liver oil and some vegetable oils.

USES: Component of Antigen Formulation (AF) and Syntex Adjuvant Formulation (SAF), present in final concentration of 5% w/v with antigen. Constitutes the oil component of the emulsion. A metabolizable oil, used in cosmetics, topicals, and as a vehicle for lipophilic drugs.

APPEARANCE: Clear oil.

MOLECULAR WEIGHT: 422.83

RECOMMENDED STORAGE: Airtight container at room temperature.

CHEMICAL/PHYSICAL PROPERTIES: Stable to air and oxygen. Readily soluble in organic solvents, slightly soluble in alcohol. Specific gravity 0.807–0.810 at 20°C.

INCOMPATIBILITY: None found.

SAFETY:

- Christian, M. S., 1982, Final report on the safety assessment of squalane and squalene, *J. Am. Coll. Toxicol.* 1:37–56.

ADJUVANT PROPERTIES: Squalane itself is not an adjuvant. See monographs on SAF-1 and AF.

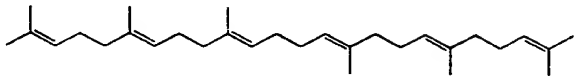
- Lidgate, D. M., and Byars, N., Chapter 12, this volume.

CONTACT(S): Supplied by several companies. For adjuvant use contact: Thomas Ryskamp, IDEC Pharmaceuticals Corporation, San Diego, CA 92121, Ph: 619-550-8500; Fax: 619-550-8750; Internet: tryskamp@idec.com. Also: Deborah M. Lidgate, Syntex Research, Palo Alto, CA 94304, Ph: 415-852-1887; Fax: 415-852-1784.

COMPONENT/ADJUVANT NAME: Squalene

OTHER NAME(S): Spinacene; Supraene; 2,6,10,15,19,23-hexamethyl-2,6,10,14,18,22-tetracosahexaene

STRUCTURE:



SOURCE: Found in shark liver oil and some vegetable oils. Intermediate in the biosynthesis of cholesterol.

USES: Bactericide, intermediate in the manufacturing of pharmaceuticals, component of MF59 emulsion formulation, constitutes the oil component of the emulsion.

APPEARANCE: Clear oil, colorless. Faint, agreeable odor.

MOLECULAR WEIGHT: 410.7

RECOMMENDED STORAGE: Store in a cool place.

CHEMICAL/PHYSICAL PROPERTIES: A metabolizable oil. Practically insoluble in water, highly soluble in organic solvents, may become viscous on absorbing oxygen. Specific gravity 0.858. Bp 285°C/25mm.

INCOMPATIBILITY: Avoid oxidizers.

SAFETY: May be harmful by inhalation, ingestion, or percutaneous adsorption. Oral LD_{50} 5 g/kg, i.v. LD_{50} 1.8 g/kg.

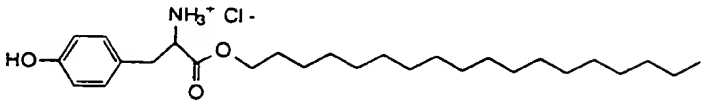
- Christian, M. S., 1982. Final report on the safety assessment of squalane and squalene. *J. Am. Coll. Toxicol.* 1:37–56.

ADJUVANT PROPERTIES: Squalene itself is not an adjuvant. See monograph on MF59.

- Sanchez-Pestador, L., *et al.*, 1988. The effect of adjuvants on the efficacy of a recombinant herpes simplex glycoprotein vaccine. *J. Immunol.* **141**:1720–1727.
- Van Nest, G. A., *et al.*, 1992. Advanced adjuvant formulations for use with recombinant subunit vaccines, in: *Vaccines 92* (F. Brown, R. M. Chanock, H. S. Ginsberg, and R. A. Lerner, eds.), Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, p. 57.
- Ott, G., *et al.*, Chapter 10, this volume.

CONTACT(S): Supplied by several companies. For example, Sigma Chemical Company, Ph: 800-325-3010. For vaccine formulation use: Gary Van Nest, Chiron Corp., Emeryville, CA, Ph: 510-601-2965; Fax: 510-601-2586.

COMPONENT/ADJUVANT NAME: Stearyl Tyrosine
OTHER NAME(S): Octadecyl tyrosine hydrochloride
STRUCTURE:



SOURCE: Chemical synthesis from tyrosine and stearyl alcohol (octadecanol).

- Penney, C. L., *et al.*, 1985, A simple method for the synthesis of long-chain alkyl esters of amino acids. *J. Org. Chem.* **50**:1457–1459.

USES: Primary vaccine adjuvant with minimal immunostimulatory properties. Some use in allergy desensitization therapy. Biocide.

APPEARANCE: White, amorphous free-flowing, odorless powder.

MOLECULAR WEIGHT: 470.14 (hydrochloride salt)

RECOMMENDED STORAGE: Store solid at room temperature. Aqueous suspensions may be stored at pH 4.0–7.5 at 4°C for several years.

CHEMICAL/PHYSICAL PROPERTIES: Sharp melting point (171–3°C). Insoluble (<0.01%) at neutral and alkaline pH; soluble at low pH in hot mineral acid.

INCOMPATIBILITY: Incompatible with strong base.

SAFETY/TOXICITY: Nontoxic up to 2500 mg/kg in many animals, including primates. Nonpyrogenic. No adjuvant arthritis (rats). No damage at site of injection (cats). Biodegradable.

ADJUVANT PROPERTIES: "Organic equivalent" of aluminum hydroxide, with likely carrier depot effect; adjuvancy similar to aluminum hydroxide with bacterial vaccines; superior to aluminum hydroxide with viral vaccines. Favorable isotype distribution. Biocompatible.

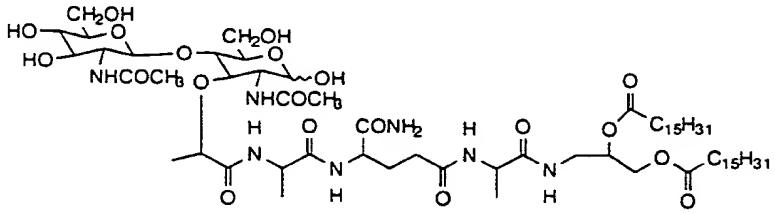
- Penney, C. L., *et al.*, 1993, Further studies on the adjuvanticity of stearyl tyrosine and ester analogues, *Vaccine* **11**:1129–1134.
- Penney, C. L., *et al.*, 1994, Further studies on the adjuvanticity of stearyl tyrosine and amide analogues. *Vaccine* **12**:629–632.
- Penney, C., Chapter 26, this volume.

CONTACT(S): Dr. Christopher L. Penney, BioChem Therapeutic, Inc., Laval, Quebec, Canada H7V 4A7, Ph: 514-978-7811; Fax: 514-978-7777.

COMPONENT/ADJUVANT NAME: Theramide™

OTHER NAME(S): *N*-acetylglucosaminyl-*N*-acetylmuramyl-L-Ala-D-isoGlu-L-Ala-dipalmitoxy propylamide (DTP-DPP)

STRUCTURE:



SOURCE: Synthetic.

USES: The drug compound is a potent macrophage activator and adjuvant. It induces IL-6, IL-12, TNF, IFN-γ, and relatively lesser quantities of IL-10. The compound preferentially induces cellular immunity. When reconstituted, it spontaneously forms liposomes in which lipopeptides may be incorporated.

APPEARANCE: White, odorless powder.

MOLECULAR WEIGHT: 1315.84

RECOMMENDED STORAGE: Stable as a lyophilized powder or in solution at room temperature for 5 years in saline or PBS at pH 7.4.

CHEMICAL/PHYSICAL PROPERTIES: Amphoteric molecule soluble in chloroform:methanol (7:3), and tert-butanol.

INCOMPATIBILITY: Avoid strong acids or bases.

SAFETY/TOXICITY: Human Phase I clinical trials at 200 $\mu g/m^2$ to 1000 $\mu g/m^2$ i.v. weekly have been initiated.

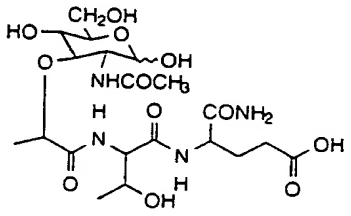
ADJUVANT PROPERTIES: The compound augments both cellular and humoral immunity and is active in murine models of CMV.

CONTACT: Gerald J. Vosika, M. D., ImmunoTherapeutics, Inc., Fargo, ND 58104, Ph: 701-232-9575; Fax: 701-237-9275.

COMPONENT/ADJUVANT NAME: Threonyl-MDP

OTHER NAME(S): Termurtide™; [thr^1]-MDP; *N*-acetyl muramyl-L-threonyl-D-isoglutamine

STRUCTURE:



SOURCE: Synthetic.

- Jones, G. J., *et al.*, Novel immunological adjuvant compounds and methods of preparation thereof. Syntex, U.S.A., U.S. Patent #4,082,735.

USES: Threonyl-MDP is included in adjuvant formulations as a primary adjuvant component.

APPEARANCE: White to off-white, odorless powder.

MOLECULAR WEIGHT: 522.5

RECOMMENDED STORAGE: The powdered drug substance should be stored dessicated at or below 25°C. For optimal stability, solutions (0.5–10 mg/mL) of threonyl-MDP should be formulated between pH 3.5 and 5.5; under this condition, a 2-year shelf life at 25°C can be expected. Solutions of threonyl-MDP formulated in a broader pH range of 1.5 to 7.5 show a 2-year shelf life if stored at 5°C.

- Powell, M. F., *et al.*, 1988, Formulation of vaccine adjuvant muramyldipeptides. 2. Thermal reactivity and pH of maximum stability of MDP compounds in aqueous solution, *Pharm. Res.* 5:528.

CHEMICAL/PHYSICAL PROPERTIES: Threonyl-MDP has an aqueous solubility of >600 mg/mL. The pKa of the isoglutamine carboxylic acid is 4.3. The compound is very hygroscopic and found to deliquesce at ≥ 68% relative humidity.

INCOMPATIBILITY: Avoid strong bases.

SAFETY/TOXICITY: At therapeutic doses, no safety concern is anticipated. The dose is indication dependent, but a guideline is 0.05–1% (w/w), with an injection volume of ~1 mL.

ADJUVANT PROPERTIES: Threonyl-MDP induces the production of a cascade of cytokines, including IL-1α, IL-1β and IL-6. Responding lymphocytes release IL-2 and IFN-γ. The latter increases the production of antibodies of certain isotypes, including IgG2a in the mouse. This isotype, and the homologous IgG1 in primates, interacts with high affinity Fcγ receptors, so that the antibodies can function efficiently in opsonizing viruses and other infectious agents for uptake by phagocytic cells.

- Allison, A. C., and Byars, N. E., 1992, Syntex Adjuvant Formulation, *Res. Immunol.* **143**:519–525.
- Allison, A. C., and Byars, N. E., 1986, An adjuvant formulation that selectively elicits the formation of antibodies of protective isotypes and of cell-mediated immunity, *J. Immunol. Methods* **95**:157–168.
- Lidgate, D. M., and Byars, N., Chapter 12, this volume.

CONTACT(S): Deborah M. Lidgate, Syntex, Palo Alto, CA 94304, Ph: 415-852-1887; Fax: 415-852-1784.

COMPONENT/ADJUVANT NAME: Ty Particles

OTHER NAME(S): Ty-VLPs (Virus Like Particles)

STRUCTURE: Amino acids 1–381 of the p1 protein encoded by the yeast retrotransposon Ty, followed by a unique restriction site for the insertion of foreign sequences and a translational stop codon.

Sequence of the p1 Ty protein:

MESQQLSQHSPISHGSACASVTSKEVHTNQDPLDVSASKTEECEKASTKANSQ
QTTTPASSAVPENPHHASPQTAQSHSPQNGPYPQQCMMTQNQANPSGWSFYG
HPSMIPYTPYQMSPMYFPPGPQSQFPQYPSSVGTPLRTPSPESGNTFTDSSSADS
DMTSTKKYVRPPPMLTSPNDFPNWVKTYIKFLQNSNLGGIIPTVNGKPVRQITD
DELTFLYNTFQIFAPSQFLPTWVKDILSVDYTDIMKILSKSIEKMQSDTQEANDI
VTLANLQYNGSTPADAFETKVTNIIDRLNNNGIHINNKVACQLIMRGLSGEYKF
LRYTRHRHLNMTVAELFLDIHAIYEEQQGSRNSKPNYRRNPSDEKNDSRSYTN
TTKPKAGS K*

SOURCE: Recombinant protein produced from *Saccharomyces cerevisiae*. Purified by filtration and chromatography techniques.

USES: As a carrier protein for expressing foreign antigens. Hybrid Ty particles induce cell-mediated immunity (without additional adjuvant) and humoral immunity (with aluminum hydroxide).

APPEARANCE: Clear aqueous solution.

MOLECULAR WEIGHT: Monomer 42,000

RECOMMENDED STORAGE: Store purified Ty particles at –20°C. Particles formulated with aluminum hydroxide should not be frozen, and can be stored at 4°C for 1–2 years.

CHEMICAL/PHYSICAL PROPERTIES: Approximately 300 monomers assemble to form a Ty particle.

INCOMPATIBILITY: Avoid contact with proteases.

SAFETY/TOXICITY: No systemic toxicity observed in human Phase I clinical trials (maximum dose 0.5 mg/subject, administered 4 times).

ADJUVANT PROPERTIES: Ty particles present antigen in a polyvalent, particulate form. Cytotoxic T lymphocytes are induced in the absence of any other adjuvant formulations.

- Adams, S. E., *et al.*, 1987, The expression of hybrid Ty virus-like particles in yeast, *Nature* **329**:68–70.
- Layton, G. T., *et al.*, 1993, Induction of HIV-1 specific cytotoxic T-lymphocytes in vivo by immunization with hybrid HIV-1: Ty virus-like particles, *J. Immunol.* **151**:1097–1107.
- Adams, S., and Kingsman, A., Chapter 34, this volume.

CONTACT(S): Dr. Sally E. Adams, British Biotech., Oxford, OX4 5LY, UK, Ph: 44-1235-551151; Fax: 44-1235-551155.

COMPONENT/ADJUVANT NAME: Walter Reed Liposomes

OTHER NAME(S): Liposomes containing lipid A adsorbed to aluminum hydroxide, [L(Lipid A + Antigen) + Alum]

STRUCTURE: Phospholipids: dimyristoyl phosphatidylcholine and dimyristoyl phosphatidylglycerol; cholesterol, Lipid A: from *Salmonella minnesota* R595, heterogeneous mixture of structures, molecular weight ranging from 1400 to 1800 depending on number of fatty acids and phosphate groups present. aluminum hydroxide gel.

SOURCE: Phospholipids and cholesterol are obtained in pure form, GMP grade, from Avanti Polar Lipids, Inc. Native lipid A, prepared by acid hydrolysis of the lipopolysaccharide of *S. minnesota* R595, is obtained from List Biological Laboratories. Monophosphoryl lipid A is obtained from Ribi ImmunoChem. Aluminum hydroxide gel is Alhydrogel or Rehydragel LV.

USES: Liposomes provide a vehicle for delivery of antigen to the immune system and also a mild adjuvant activity, but liposomes containing lipid A provide a very potent adjuvant activity. Adsorption of liposomes containing lipid A to aluminum hydroxide gel contributes additional strong adjuvant activity with many antigens. Liposomes containing lipid A have been shown to induce both humoral and cell-mediated immunity.

APPEARANCE: White, opalescent particulate suspension.

MOLECULAR WEIGHT: Equal to the sum of the molecular weights of the components used in the formulation, e.g., antigen molecular weight will vary with the vaccine formulation.

RECOMMENDED STORAGE: Store liquid liposome formulations at 4–6°C. Lyophilized liposomes prior to reconstitution with antigen may be stored at either 4–6°C or –20°C. Liposomes in the liquid form reconstituted with antigen are stable for at least 1–2 years.

CHEMICAL/PHYSICAL PROPERTIES: Liposomes are stable at pH from 1 to 10. Solubility and stability will depend on the antigen encapsulated. Phospholipids, cholesterol, and native lipid A are soluble in chloroform. Monophosphoryl lipid A is soluble in chloroform–methanol 9:1. All liposomal components are stable in organic solvents for at least 1 year when stored at –20°C.

INCOMPATIBILITY: None found.

SAFETY/TOXICITY: Liposomal vaccine formulations have been administered to humans in four Phase I or Phase I/IIa clinical trials (three containing recombinant antigens derived from the *Plasmodium falciparum* sporozoite and one containing gp120 derived from the envelope of HIV). The vaccine formulations used in all four trials passed all preclinical safety and toxicity tests and no adverse side reactions have been observed.

- Fries, L. F., *et al.*, 1992, Liposomal malaria vaccine in humans: A safe and potent adjuvant strategy, *Proc. Natl. Acad. Sci. USA* **89**:358–362.

ADJUVANT PROPERTIES:

- Alving, C. R., and Richards, R. L., 1990, Liposomes containing lipid A: A potent nontoxic adjuvant for a human malaria sporozoite vaccine, *Immunol. Lett.* **25**:275–280.
- Verma, J. N., *et al.*, 1992, Adjuvant effects of liposomes containing lipid A: Enhancement of liposomal antigen presentation and recruitment of macrophages, *Infect. Immun.* **60**:2438–2444.
- Alving, C. R., *et al.*, 1992, Liposomes containing lipid A as a potent non-toxic adjuvant, *Res. Immunol.* **143**:249–251.
- Alving, C. R., *et al.*, 1993, Novel adjuvant strategies for experimental malaria and AIDS vaccines, *Ann. N.Y. Acad. Sci.* **690**:265–275.

CONTACT(S): Dr. Carl Alving, Dr. Nabila Wassef and Dr. Roberta Richards, Department of Membrane Biochemistry, Walter Reed Army Institute of Research, Washington, DC 20307-5100, Ph: 202-782-3248; Fax: 202-782-0721.

3. ACKNOWLEDGMENTS AND UPDATE SUMMARY

This compendium would not have been possible without the enormous support we received from vaccinologists, formulators, immunologists, and others worldwide. We are indebted to the following contributors for both general guidance and, in many cases, sending us up-to-date information on specific vaccine adjuvants and components: Sally Adams (British BioTechnology, Oxford, UK); Carl Alving (Walter Reed Army Institute of Research, Washington, DC); Françoise Audibert (Vacsyn SA, Paris, France); Gail Barchfeld (Chiron Corp., Emeryville, CA); Bob Baughman (Emisphere Technologies, Hawthorne, NY); Joan Bell (Genetics Institute, Cambridge, MA); Diana Boraschi (Dompè S.p.A., L'Aquila, Italy); Eugene Bortnek, (Seppic, Fairfield, NJ); James N. Brewer (University of Strathclyde, Glasgow, UK); Robert Brey (Vaxcel, Inc., Norcross, GA); Louis Chedid (Vacsyn SA, Paris, France); Robert Chesnut (Cytel Corp., San Diego, CA); Linda C. Clarke (Washington Research Foundation, Seattle, WA); Jeffrey Cleland (Genentech, Inc., S. San Francisco, CA); John Clements (Tulane University, New Orleans, LA); Peter D. Cooper (John Curtin School of Medical Research, Canberra, Australia); Peter Cooper (CC Biotech, Poway, CA); Raymond Daynes (University of Utah, Salt Lake City, UT); Phillipe DeLafaire (Seppic SA, Paris, France); David Dodds (Schering Corp., Bloomfield, NJ); Friedrich Dorner (Immuno AG, Ortha Donau, Austria); Johann Eibl (Immuno AG, Vienna, Austria); Vincent Ganne (Seppic SA, Paris, France); Maury Gately (Hoffmann–LaRoche, Nutley, NJ); Karen Goldenthal (FDA, Washington, DC); Michael Goodman (Scripps Research Institute, La Jolla, CA); Geoffrey Gorse (Saint Louis University, St. Louis, MO); Susan Gould-Fogerite (UMDNJ, NJ Medical School, Newark, NJ); Rajesh Gupta (Mass. Institute of Public Health, Jamaica Plain, MA); Nabil Hanna (IDEC Pharmaceuticals, San Diego, CA); Robert Hanson (3M Pharmaceuticals, St. Paul, MN); Andy Heath (University of Sheffield Medical School, Sheffield, UK); Stanley Hem (Purdue University, West Lafayette, IN); Cliff Hendrick (Genzyme Corp., Cambridge, MA); Luuk Hilgers (Solvay SA, Brussels, Belgium); Rodney Ho (University of Washington, Seattle, WA); Maninder Hora (Chiron Corp., Emeryville, CA); Huw Hughes (M6 Pharmaceuticals, Yonkers, NY); Robert Hunter (Emory University, Atlanta, GA); Arthur Johnson (University of Minnesota, Duluth, MN); Stephen Kaminsky (United Biomedical Inc., Hauppauge, NY); Charlotte Kensil (Cambridge Biotech, Worcester, MA); Philip B. Klepak (Reheis Inc., Berkeley Heights, NJ); Jorg Kreuter (Johann Wolfgang Goethe-Universität, Frankfurt, Germany); Lawrence Lachman (M. D. Anderson Cancer Center, Houston, TX); Robert Leather (Reheis, Inc., Berkeley Heights, NJ); Philip Ledger (Peptech (*UK*) Ltd., Cirencester, Glos, UK); Deborah Lidgate (Syntex Research, Palo Alto, CA); Amy Lim (Genentech, Inc., South San Francisco, CA); Erik B. Lindblad (Superfos Biosector a/s, Vedbaek, Denmark); Oswald Lockhoff (Bayer AG, Leverkusen, Germany); Nils Lycke (University of Göteborg, Göteborg, Sweden); Jeannine Majde (Office of Naval Research, Arlington, VA); Raphael Mannino (UMDNJ, NJ Medical School, Newark, NJ); Richard McIntosh (Genesis Tech. Group, Cambridge, MA); Richard Miller (3M Pharmaceuticals,

St. Paul, MN); Nahid Mohagheghpour (SRI International, Menlo Park, CA); Bror Morein (National Veterinary Institute, Uppsala, Sweden); Kent R. Myers (Ribi ImmunoChem, Hamilton, MT); Tattanahalli Nagabhushan (Schering Corp., Bloomfield, NJ); Marian Neutra (Harvard Medical School, Boston, MA); Tony Newton (Genzyme Corp., Cambridge, MA); Jack Nunberg (Genentech, Inc., S. San Francisco, CA); Keiko Oishi (Genentech, Inc., Japan); Albert Osterhaus (Erasmus University, Rotterdam, Sweden); Gary Ott (Chiron Corp., Emeryville, CA); Lendon Payne (VRI, Cambridge, MA); Christopher Penney (BioChem Therapeutic Inc., Quebec, Canada); Ramachandran Radhakrishnan (Chiron Corp., Emeryville, CA); Mike Ramstack (Medisorb Tech. Intl., Cincinnati, OH); Al Reisch (Sargeant, Inc., Clifton, NJ); John Rhodes (The Wellcome Research Labs, Kent, UK); Roberta L. Richards (Walter Reed Army Institute of Research, Washington, DC); Bryan Roberts (VRI, Cambridge, MA); Thomas Ryskamp (IDEC Pharmaceuticals Corp., San Diego, CA); Noemi Santiago (Emisphere Technologies, Hawthorne, NY); Paul Sleath (Immunex Corp., Seattle, WA); Christine Smart (Proteus International, PLC, Macclesfield, UK); H. Snippe (University Utrecht, Utrecht, Netherlands); Alvin S. Stern (Hoffmann–LaRoche, Nutley, NJ); Bo G. Sundqvist (Iscotec, Uppsala, Sweden); Aldo Tagliabue (Dompè S.p.A., L'Aquila, Italy); J. Terry Ulrich (Ribi ImmunoChem, Hamilton, MT); Peter van Hoogevest (Ciba Geigy, Basel, Switzerland); Gary Van Nest (Chiron Corp., Emeryville, CA); Gerald Vosika (ImmunoTherapeutics, Inc., Fargo, ND); Nabila M. Wassef (Walter Reed Army Institute of Research, Washington, DC); Robert Weissburg (Genentech, Inc., South San Francisco, CA); Stan Wolf (Genetics Institute, Cambridge, MA).

We also thank Jessica Burdman for technical editing and organizational aspects, and Mili Chin for secretarial assistance. Tom Patapoff played a key role in translating several summaries from "unusual" format to Mac or PC format. A special thanks to Rodney Pearlman for supporting this work, and to Igor Gonda for his help. A great deal of adjuvant support and advice was also provided by Phil Berman, Tim Gregory, and Jack Obijeski, all of Genentech.

Finally, it is our goal to update this compendium in 1996–1997. Our goal is to present an accurate listing of vaccine adjuvants available for use in basic research, and preclinical and clinical evaluation. In some cases, we have shown only a few of the possible contacts or suppliers available for a particular adjuvant, of consideration in keeping this compendium as short as possible. Many suppliers often exist for a particular adjuvant and its components. If you would like to update a monograph in the next edition of this compendium, or contribute a new one, please send your information to:

Dr. Michael F. Powell
Genentech, Inc. MS#82
460 Pt. San Bruno Blvd.
South San Francisco, CA 94080
Ph: 415-225-1389
Fax: 415-225-2866
E-mail: powell.mike@gene.com